

The motor-car as black sheep

Britain's Royal Commission on Environmental Pollution has joined the lobby against the motor-car without paying enough attention to the political obstacles to the pursuit of draconian policies.

ONE measure of the public influence of Britain's Royal Commission on Environmental Pollution is that the price of shares in automobile manufacturers on the world's stock markets has not precipitously fallen in the past two weeks, since the appearance of the commission's report on transport and the environment (see *Nature* 372, 9; 1994). Such sangfroid in the face of a call for extra taxes to double European motor-fuel prices in a decade is less likely to be a sign of corporate folly than a proof that Europe's voters are unconvinced. For one thing, they do not believe that governments who look to them for re-election would have the cheek. And those who have read the commission's report will recognize that its earnest case for cutting back on gasoline consumption is as much motivated by trendy distaste for motor-cars as by a wish to be politically persuasive.

This royal commission has a unique place in British public life, in that it is the only body licensed in perpetuity to give independent and public advice in a general area of public policy. (The commissions on historical documents and, since last week, on MPs' conduct are more narrowly constrained.) The commission has hitherto been level-headed in the advice it gives. Its new report is intellectually more ambitious than the 17 earlier documents in the series: manfully, it sets out to "internalize the externalities", as environmentalists are fond of urging, tracking down many of the hidden costs of various forms of transport and declaring that they should be made explicit components of the cost thereof. The weakness in the argument is also familiar; not all the externalities make their appearance.

The case against road transport is familiar. It is an environmental nuisance (noise), a thief of land (for roads and clover-leaves), a source of air pollution (carbon dioxide, tropospheric ozone, nitrogen oxides, lead and mildly carcinogenic benzene) and a notorious cause of fatality. The other side of the coin is less often made explicit, but is easily rehearsed: by allowing people to make journeys at their own volition, at times that suit them and between destinations whose positions on the map are defined to within a metre or thereabouts, motor-cars have become increasingly the preferred form of surface transport.

Prosperity

It is therefore no surprise that the first few chapters of the commission's new report are replete with evidence (not only from Britain) that the use of motor-cars and other personal

vehicles is correlated with prosperity (national and personal) and that it is still booming. For what it is worth, the report is also packed with invaluable information about the vagaries of the British transport system. But the essence of the political difficulty the commission has not faced is that the people who enjoy the benefits of personal transport are a very substantial subset of those who complain about the nuisance, the loss of land, the air pollution and the fatalities on the roads. The implication that the former are fecklessly indifferent to the environmental nuisance they cause, and that the latter are percipient modernists, strains credulity; mostly, they are the same, which is the problem.

Remedies

In the circumstances, the remedies the commission advocates are but crude solutions of an admittedly difficult problem. Thus the case for the draconian tax on motor-fuels advocated in the report derives almost exclusively from greenhouse arguments. Surface transport in Britain is estimated to have been responsible in 1990 for the emission of 30 million tonnes of carbon as carbon dioxide, or for about 21 per cent of Britain's total emission of that greenhouse gas. Moreover, transport emissions are increasing; they almost doubled in the 20 years to 1990 and are likely to double again in a further 30 years as more people gratify their wish to become liberated by access to personal transport.

The commission notes that this state of affairs might be reconciled with British obligations under the Rio convention on global warming if the expected increase of transport emissions were offset by reductions elsewhere, but it then offers the opinion that "we consider that transport must take its share of the reductions" and goes on to recommend that the British government should aim at reducing emissions from surface transport to 80 per cent of the 1990 level in the coming quarter of a century. That is the rabbit from a hat from which many of the other recommendations follow logically — the cancellation of all British road-building schemes for which contracts have not yet been let, for example.

It is a matter for regret that a potentially influential report should so have stubbed its toe on political fallacy almost at the outset (by the end of the third of fourteen chapters). British voters (like those of other nationalities) are not yet satiated with personal transport. Moreover, faced with a need



democratically to opt for lower greenhouse emissions, they would almost certainly prefer that reductions should come from some other source, however inconvenient and costly the alternatives might be. It is not, after all, that the uses people make of motor-cars are frivolous. To rural people, cars have become literally necessities. To large sections of most advanced societies, they are also means to personal efficiency that will never be entirely replaced by the benefits of telecommunications.

Balance

It is entirely right that the externalities of surface transport should be internalized, but the commission's figures suggest that Britain has magically arranged that the overall balance is about right. In the financial year just past, the British government collected about £20.4 billion from transport taxes, compared with an estimate of between £16.9 billion and £25.2 billion for the environmental costs of road transport (including the costs incurred in building and maintaining roads and the cost of accidents). The immediate objective now should be more accurately to match marginal costs. That argues for a readjustment of the tax burden. The British government is already tempted by the idea of schemes for road pricing in congested cities and motorway tolls. In equity, bearing in mind the interests of rural people (and the circumstance that the environmental costs of rural motor-cars are relatively low), those revenues should be used to reduce, not to increase, the costs of motor-fuel.

That does not imply that there are not serious problems ahead. One is occasioned by the probable secular increase of the price of crude oil as the accessible (and thus cheap) reserves are progressively exhausted. Unless alternative fuels (natural gas or electrolytic hydrogen seem the best bets in Europe) or means of propulsion (electric vehicles) become economic and reliable, it seems inevitable that there will be a secular decline in the availability (and the benefits) of personal motorized transport. Teleworking will soften the hardships thus created, but not completely. So the cost of social deprivation in rural communities will become one of the externalities to be included in the consideration of future transport policies.

There remains the greenhouse problem. Britain, to which the bulk of the commission's argument is addressed, has a reasonable record on compliance with the European Union's target of restoring greenhouse emissions to 1990 levels by 2000, but that is the easy part. Compliance has been assisted by the recession, while the target is insufficient for the long run. Given what appears to be its new-found ambition for making quantitative studies, the commission could do worse than embark on a study of alternative ways of reaching alternative targets, taking full account of the social and environmental consequences of those developments. But it should do what it can to suppress its populist animus against the motor-car, which has not yet become the icon of a profligate age the commission supposes it to be. □

Peace in Ireland?

The British and Irish governments could settle some contentious issues quickly.

SINCE the declaration by the Irish Republican Army (IRA), relayed to the world by the political party Sinn Féin, that military action would be halted, the British and Irish governments have been working diligently but cautiously on the document they hope may lead to constitutional change. The British are understandably the more cautious; the government's commitment to the people of Northern Ireland is explicit and unambiguous; there will be a united Ireland only if a majority of the people in the north elect for such a change. Nobody can complain at that; what else is democratic self-determination? But there is much else that can and should be done short of a united Ireland, to bring the communities of the Republic of Ireland and of Northern Ireland together.

The old links between north and south are much stronger than they have seemed in the last 25 years, since the first shootings at Londonderry. Both collaborate in mounting a joint team to compete in rugby football, for example, while there is still a Royal Society of Ireland in republican Dublin. But these are mostly historical accidents in a country in which history has a bad name. The more immediate need is for common institutions that are both modern and productive of benefits that will quickly become apparent. For many years, this journal has been urging that higher education and research provide a field that could function as cross-border cultural cement without prejudicing the great constitutional questions still unresolved. This is surely the time when the opportunities should be seized.

There are several things to do, among which the chief should be an open declaration that the two university systems will in future be planned in common and financed through a common mechanism (to which both governments contribute). Because of the links between the universities of Northern Ireland and those in the rest of the United Kingdom, that would imply a direct link into the rest of the university system throughout the British Isles. On the research side, there should be an understanding that academics from either north or south could apply for research support from any research council in the same territory. Initially, the British government would have to bear the major part of the cost, but that should not persist indefinitely. Unified research funding should quickly persuade the republic's government that it must (in the interests of its young people) pay more attention to research.

Meanwhile, there is a particular issue to decide. The University of Ulster has a plan to build a new campus in Belfast in a run-down region of the city straddling what is called the "peace-line" (more accurately, it is a no man's land) between the loyalist and nationalist communities. It is an imaginative scheme. It would strengthen an already innovative university. And it would help to cement what is still an uneasy peace. Why not do that now? □

Space scientists protest at decision to scrap plans for Pluto mission

Washington. Daniel Goldin, administrator of the US National Aeronautics and Space Administration (NASA), has decided to scrap plans for a spacecraft mission to Pluto, claiming they are too expensive. His decision has not only sent frustrated project designers back to the drawing board but also casts doubt on NASA's willingness to mount any expedition to the last uncharted planet in the near-term future.

Goldin told last week's meeting of the American Astronomical Society's Division for Planetary Science (DPS) in Washington that \$600 million was "way, way too much for one mission". Later in the meeting, William Piotrowski, head of NASA's Solar System exploration division, told a workshop that "the current concept for the Pluto mission is dead".

The decision is a major blow to scientists and engineers who have been working on plans for the Pluto Fast Fly-By (PFF), as they have consistently met cost and performance targets set by Goldin. Indeed, it was partly Goldin's own interest in the Pluto concept that elevated it within the pecking order of proposed NASA planetary missions two years ago (see *Nature* 358, 701; 1992). The mission had been targeted for a "new start" in NASA's 1998 budget.

The PFF concept, which Goldin has touted as a showpiece for his "better, cheaper, faster" philosophy, called for sending two identical spacecraft on a fast, direct route to the planet, with a launch in 2001 and arrival at Pluto in 2008. Mission designers relied heavily on advanced technology, much of it still being developed, to reduce the spacecraft's weight and boost performance.

They also stripped the science payload down to a 7-kg package with only four science instruments. The PFF would have been lighter and more capable than the Clementine spacecraft that mapped the Moon earlier this year and would have used two-thirds the number of people for mission operations during the Pluto encounter.

More than a year ago, Goldin assured PFF team members that if they brought their costs under \$750 million, he would back the project. He also encouraged them to solicit

Russian participation. The team met both targets by trimming the mission's price to \$580 million, including a launch on a Russian Proton rocket and a small Russian probe dropped into Pluto's atmosphere.

The project also received high marks from an independent review team recruited

comet mission, set for launch in 2003. This would partly make up for a NASA comet rendezvous mission cancelled by Congress two years ago.

The PFF project suffers from not having as large a constituency as these other missions. Pluto was not listed as a priority in a recent report by the National Research Council's Committee on Planetary and Lunar Exploration (COMPLEX), which recommended focusing on comets, Mars, Jupiter and a search for planets around other stars instead. But NASA's scientific advisory committees have supported the Pluto mission, as have the agency's senior science managers. The idea has also gained support from the press and public.

Goldin told his audience at DPS that he sympathized with those at the NASA Jet Propulsion Laboratory (JPL) who were keen to get the Pluto mission started, adding "I don't want them to feel it's over". Huntress, too, advises patience, and says he believes some kind of Pluto mission will be approved eventually, perhaps even in time for a 2001 launch — but the PFF team has been told that study money for such a mission is being cut.

Cutting the cost to \$250 million, which appears to be Goldin's target, is a "very tall order", says Alan Stern of the Southwest Research Institute in San Antonio, Texas, who led the NASA advisory group that shaped the objectives for the PFF mission.

Stern points out that when mission reserves and other built-in costs are deducted from the \$580 bill, it leaves only \$390 million for two spacecraft — including the launch — roughly equivalent to the price of two \$150 million Discovery missions, which do not include launch.

Richard Terrile, PFF's project scientist at JPL, says the cost could be trimmed to between \$300 million and \$380 million if NASA settled for sending only one spacecraft (outside advisory groups have advised against that strategy as too risky), and took a slower route using a gravity boost from Jupiter. He is sceptical that the price can come down much lower.

Members of the PFF team say they are less bothered by the cost-cutting than by the uncertainty about what Goldin wants. Although he has admonished space engineers to save money by using "revolutionary technology" for the Pluto mission, he has not spelled out what that means. "They [NASA] want to fly something that's higher-tech [than PFF]," said Terrile at the DPS Pluto workshop, "even though nothing flying on this mission has ever been flown before."

Tony Reichhardt

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Little Brother: The small size of the Pluto spacecraft (right), compared here to the Cassini spacecraft, has challenged the ingenuity of NASA engineers.

by Goldin from the same Pentagon organization that funded Clementine. But Goldin is now saying that this is not sufficient. As Wesley Huntress, NASA's head of space science, admitted at last week's DPS meeting, "the goalposts have moved".

The decision has raised questions about Goldin's mercurial management style. Some admire it, saying it stimulates innovation and efficiency; others find it disruptive. Goldin defends his decision by explaining that NASA has less money now than two years ago, when he first became excited about the project. "I want to keep the heat on to get the cost down," he says.

The agency also has higher priorities in planetary science, starting with a new line of low-cost Discovery missions expected to begin in 1996 (winners will be chosen in January from among 28 proposals). In addition, NASA science managers recently added a new \$120-million project to their wish list, namely participation in the European Rosetta

Economist tipped as German research minister

Munich. Klaus Töpfer, Germany's environment minister for the past seven years and formerly a professor of economics, is being tipped to become Germany's next research minister when the new cabinet is announced at the end of this month.

Töpfer, who is 56, is a Christian Democrat whose party wanted to hold on to

power in last month's general election. If his appointment is confirmed, Töpfer could inherit broader responsibilities than his predecessor Paul Krüger, an east German, as the federal research and education ministries may be merged into what is being informally called a "ministry of the future".

A. A.

NIH files counter-patent in breast cancer gene dispute

London. The US National Institutes of Health (NIH) has decided to challenge the patent filed last month by the University of Utah and Myriad Genetics on the breast cancer gene BRCA1 by filing its own patent application on the same gene.

According to Harold Varmus, the director of NIH, the organization has taken this action because the original application, based on the work of a team headed by Mark Skolnick, omits the names of researchers from the National Institute of Environmental Health Sciences (NIEHS), even though they are on the paper published in *Science*.

In contrast, the NIH application includes the names of two NIEHS researchers, as well as seven other key scientists from the university and Myriad. Negotiations are now taking place between the two sides, one possible outcome being an amalgamation into a single application.

Researchers at Myriad Genetics, which holds an exclusive license with the university to any patents resulting from their joint research, say that NIEHS scientists — J. Carl Barrett and Roger Wiseman — were excluded because their contribution was not considered central to the work.

But Varmus says that the main reason for filing a separate patent is his fear that, without the name of NIEHS researchers on it, a patent application would be ruled invalid. "It is in everyone's interests to have a clear and correct patent," he says. "It creates chaos to have patents overturned."

In addition to the intellectual argument over who should be credited with the discovery of the gene, Varmus also acknowledges that NIH's name on the patent will give the agency a say in how the licensing of the gene should be handled — and how any royalties should be split.

Before the announcement of its patent application, for example, Varmus had received a letter from Representative Ron Wyden (Democrat, Oregon), questioning

the omission of the NIEHS researchers from the Utah application and claiming that this would deprive US taxpayers of any direct return on their investment in the research.

Varmus claims that, even if the NIH is eventually acknowledged as a co-owner of the patent, this will have little bearing on the way that it is licensed. But he declined to comment on the details of the application.

In particular, he will not say whether its coverage is as broad as that of the Utah/Myriad patent, which claims the rights to the BRCA1 gene and to all possible mutations that can give rise to the disease.

Even researchers at Myriad are uncertain whether the US Patent Office will accept an application with such broad coverage. They justify the inclusion of the gene (and the mutations) as part of the knowledge required to develop a diagnostic kit.

But the application has angered other researchers in the field. If granted, it would give the University of Utah and Myriad the rights to any other mutation that may be discovered, even though this could be the result of many years of work by scientists and with families that have no link with Utah.

"I have a number of families under my care," says Bruce Ponder of the University of Cambridge, whose own group had also been involved in the race to discover BRCA1, and is still searching for mutations in the gene. "If I wish to offer them a prediction service based on the techniques I have developed, I do not see why I should pay a license either to Myriad or the NIH to do that."

Partly in reaction to the way that the BRCA1 patent is being handled, a number of research teams in both the United States and Europe currently engaged in the search for mutations have decided to form themselves into a loose-knit consortium that will agree to share family data and primers between themselves. "The idea is to make our efforts complementary and not competitive," says Ponder. □

Varmus speaks out on need to boost clinical research

Washington. Harold Varmus, the director of the US National Institutes of Health (NIH), last week asked the American Association of Medical Colleges to suggest an ombudsman to help him resolve a "crisis of confidence" in US clinical research.

By clinical research, Varmus said he meant that in which doctor and patient remain in the same room, rather than a clinician working with, say, a tissue sample. He said that concern about clinical research has existed since the 1970s, but is more pressing today because of the growth in the clinical applications of basic research.

Since his appointment as head of the NIH, Varmus says he has received a barrage of letters about clinical research, some worrying that the discipline is going to waste, others concerned that it is reviewed less favourably by NIH than basic research.

Varmus says that he has yet to decide whether to appoint a top-level panel for clinical research. But he has already appointed a committee to assess how fairly clinical research is peer reviewed, following complaints that MDs fare less well than PhDs in applications for NIH research funds, and that study sections are prejudiced against the high cost of patient-orientated research.

This committee will report in December. But Varmus says there is no evidence of prejudice against MDs and denies that patient-orientated research is losing out because of its cost. Nevertheless, the NIH finds it difficult to recruit clinicians to serve on study sections reviewing clinical research.

The committee assessing peer review for clinical research has also been looking at the training of MDs. Varmus says he is concerned by reports that medical schools teach laboratory work rather than patient-orientated research. He advocates optional courses on how to carry out clinical research which would teach prospective investigators about the role of the institutional review boards that decide whether protocols conform to guidelines and the importance of proper consent forms and of monitoring patients for adverse effects.

Both of the latter issues have recently been in the spotlight. For example, health activists and congressional committees criticized the consent form drawn up by the National Surgical Adjuvant Breast and Bowel Project for not spelling out fully the risks associated with taking the anticancer drug tamoxifen in a breast cancer prevention trial (see *Nature* 369, 515; 1994).

The project also came under fire for not properly monitoring the data collected by investigators. The NIH is now conducting a survey of how grant administrators monitor clinical trials.

Helen Gavaghan

Microbial collections 'need a policy'

London. The 11 repositories that make up the United Kingdom's microbial culture collection should be brought together under the aegis of the Biotechnology and Biological Sciences Research Council to protect a "major national asset". This is the conclusion of an independent review commissioned by the Office of Science and Technology and published last week.

The review panel concluded that the diversity and geographical separation of the institutes responsible for the different collections was a major factor in produc-

ing a resource respected for the authenticity and purity of its samples.

But with nine parent organizations involved in funding and maintaining the 11 institutions, and with uncertainty hanging over their future because of government pressure to increase the effectiveness of its research efforts, the committee says that a national policy would now be "opportune".

Recommended changes include a co-ordinated development and marketing strategy. The government is considering the report's conclusions. □

India ponders the flaws exposed by plague...

Surat & Bombay. Threatened with becoming an international 'no-go' area for businessmen and tourists alike, India took Herculean measures in September to control the outbreak of pneumonic plague in Surat.

Although the glare of international attention has now turned away, it is under growing pressure to act on the lessons learnt from the outbreak, in particular the need for better hygiene and sanitation facilities and for improved epidemiological monitoring.

Within 24 hours of the first reports of plague, almost all of the one million people living in the slums of Surat, which house half the city's population, had received courses of tetracycline. Vast areas were also sprayed with flea-killing insecticides and a massive 'dragnet' was set up to identify suspected cases of the plague.

It was an "amazing" effort, says David Dennis of the US Centers for Disease Control in Atlanta, Georgia, the spokesman for a team of experts sent to India by the World Health Organization (WHO) last month to investigate the outbreak.

Nonetheless, the outbreak has exposed major deficiencies in India's capacity to

carry out the critical laboratory and epidemiological studies needed during the outbreak of a rare disease. In particular, the dragnet appears to have overwhelmed the normal system of being able to prove and document what was going on.

The Indian authorities had a highly disciplined approach to collecting patient specimens. Critics claim that the problem was what happened to them afterwards. In particular, the techniques used failed accurately to identify the plague agent, *Yersinia pestis*, and the histochemical staining used was invalid because all the isolates were contaminated. Furthermore "they didn't have the capacity to put the organism through lab animals, or to do biochemical or bacteriophage testing", says one critic.

In its preliminary report, the WHO team recommends that India "maintains the expertise needed to deal with outbreaks of new and re-emerging diseases" and that it

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Dysentery, dengue fever, malaria and tuberculosis are widespread in the slums of Surat.

strengthen relevant "surveillance, laboratory facilities and epidemiological capacities", with an implicit reference to achieving a high scientific standard.

Euvegnny Tikhomirov of WHO's division of communicable diseases points out that there is a known reservoir of plague in wild rodents in parts of India that cannot be eradicated — and that wild rodents can transmit the disease to domestic rodents.

This reservoir needs to be monitored constantly, says Tikhomirov, and people must be educated to notify health authorities when they notice domestic rats, which are highly susceptible to plague, dying suddenly.

But plague is only one of several infections endemic to the region. According to the United Nations Children's Fund (UNICEF) 300,000 children die each year of diarrhoea in urban areas of India alone, while dysentery, dengue fever, malaria and tuberculosis are widespread in the slums.

Other observers say that neglect of basic sanitation services keeps a disease time-bomb ticking under the country. Many are also concerned that whereas 'sanitation' and 'hygiene' leapt onto the national agenda during the outbreak, this interest will subside now that the international spotlight has been turned off.

"The post-plague cleanup provided a shocking indication of what could be done," says the magazine *India Today*, writing of "the rare sight of municipal authorities working overtime to remove garbage and unclog sewers". The magazine claims that effective collection is prevented by widespread corruption, racketeering and inefficiency.

Surat is the diamond capital of India. It also boasts the world's largest steelworks, has a booming carpet industry and is one of the top ten contributors to the national treasury.

But 250 of the 1,250 tonnes of garbage produced daily goes uncollected, as does 60 of the 130 million litres of sewage. Industry and economics are being put before public health, complains B. D. Parmar, director of the New Civic Hospital in Surat.

Declan Butler

... as doubts over outbreak rumble on

Surat & Delhi. Confirmation by a team of experts set up by the World Health Organization (WHO) that the results of epidemiological studies in Surat were compatible with a limited outbreak of plague has done little to dampen scepticism among Indian microbiologists about the exact nature of the disease (see *Nature* 371, 547; 1994).

"The WHO team has only confirmed our suspicions," says N. P. Gupta, former director of the National Institute of Virology in Pune and one of the microbiologists who believe the outbreak was not plague. Gupta points out that the team found no evidence for pneumonic plague outside Surat, even though half a million people fled the town during the first three days of the outbreak.

But one member of the WHO team argues that pneumonic plague is not as contagious as is often claimed. He points out that, although many pathogens spread via aerosols, pneumonic plague spreads by respiratory droplets and requires close contact. "Patients in Surat died so quickly they didn't have time to contaminate others," he says.

V. V. Maleev, another member of the WHO team, says he has seen confirmed plague cases elsewhere and that the cases in Surat were not "typical". He claims that as many as half the 54 deaths attributed to plague in Surat were due to other causes.

A national committee has been set up to investigate the outbreak, chaired by V. Ramalingaswami, former director-general of the Indian Council of Medical Research.

Its first meeting was turbulent, with many accusing the National Institute of Communicable Diseases of acting too hastily in labelling the outbreak as plague.

But one member of the WHO team argues that the uncertainty was merely the consequence of the fact that, as local laboratories used poor procedures, the original diagnosis of plague was not backed up during the outbreak itself by unequivocal identification of *Yersinia pestis* in cultures from blood, sputum and autopsied organs (see above).

David Dennis, from the US Centers for Disease Control in Atlanta, Georgia, and spokesman for the WHO team, says the WHO study was of necessity retrospective, and could not be expected to provide precise figures on the number of cases.

But Dennis says he is "100 per cent sure" that pneumonic plague occurred in Surat and bubonic plague in Beed in the state of Maharashtra.

Dennis adds that the team also detected antibodies to the fraction 1 antigen of *Y. pestis* in the serum of suspected plague patients.

The working hypothesis of the WHO team, he says, is that 'secondary' pneumonic plague was introduced to Surat by an individual suffering from bubonic plague that had developed into septicaemia and subsequently seeded the lungs. This would explain why the outbreak in Surat was not preceded by an outbreak of bubonic plague.

K.S. Jayaraman & Declan Butler

Primate groups link up to oversee supplies

Munich. Five European primate research and breeding centres, backed by the European Commission (EC) in Brussels, have set up a common network to coordinate the supply of European-bred primates of high quality to researchers and industry, to advise on reducing the number of primates used in medicine, and to develop ethical standards for the use of primates in research.

The European Primate Resources Network (EUPREN), linking five separate centres, was established last month. One of its tasks will be to compile accurate figures of the number of primates used in Europe.

A longer term aim is to increase the supply of European primates in order to avoid importing animals. But this idea remains controversial, as some animal welfare groups claim that it detracts from a commitment to reduce the number of animals used in research.

The move comes at a time when the EC is caught between pressures to reduce the use of primates in medicine and research — particularly primates caught in the wild — and the need to maintain supplies of high quality primates where their use is unavoidable.

Europe's total primate breeding capacity — based on a relatively large breeding centre in Göttingen, Germany and smaller facilities in Strasbourg, France, and Rijswijk in the Netherlands (see box) — is not much more than 500 animals per year.

But it is estimated that Europe uses 10,000 primates a year, 90 per cent by commercial companies. About one-third of this is for vaccine development and production and the rest is in drug testing and medical research.

Animals also come from breeding centres in the primates' country of origin — China for example, has many such centres — or are captured from the wild. But both animal welfare groups and scientists working with primates criticize the importation of animals because of the stress caused by long-distance travel and the im-

pact on wild populations.

They also agree that unregulated breeding conditions in the third world are unacceptable, while scientists often prefer not to work with animals caught in the wild, not only for ethical reasons, but also because of their unknown age and history of infection.

The EC's environment directorate, under a council of ministers' directive passed in 1986, has been instructed to move towards a complete ban on the import of animals caught in the wild and to ensure that as few as possible are used in research.

The EC's research directorate last year set up a Primate Vaccine Evaluation Network (PVEN) in an attempt to meet the implications of these moves. It operates in 69 countries, inspecting breeding centres and only admitting as members those which fulfil stringent conditions.

Michael Balls, director of the European Centre for Validation of Alternative Methods (ECVAM) at the EC's Joint Research Centre in Ispra, Italy, and an adviser to the environment directorate on the welfare of laboratory animals, says that before any move is made to increase the supply of European primate. Europe should accurately assess how many primates are really essential in medicine and research.

Balls points out that the purpose of the directive is to reduce the use of animals — particularly primates — in research, and argues that it is still too easy to get approval for the use of primates.

Micha Roumiantzeff from the European Vaccines Manufacturers Association, a group representing seven companies which supply two-thirds of the world's vaccines, is concerned that attempts significantly to increase the breeding of primates in Europe could be unrealistic on economic grounds.

Vaccine production and testing, he says, as well as drug testing, are regulated by authorities that demand specific species of primate and it is difficult simply to ask

companies to cut numbers or use alternatives. He argues that, as an alternative, EUPREN should, in conjunction with PVEN, become involved in regulating the importation of animals, a suggestion currently under discussion at the commission.

Roumiantzeff says companies are also worried that the commission's plans to reduce the use of animals in research could lead to a shortage of primates for vaccine production in Europe.

Continuous supplies are needed if vaccine production is not to suffer, he says, and new breeding colonies can take years to establish. Industry still relies on import of primates caught in the wild, he says, and an immediate ban would cause problems.

Alison Abbott

Reprieve for Dutch animal centre?

Munich. A primate centre run by the National Applied Research Organization (TNO) at Rijswijk in the Netherlands, which was threatened with closure two years ago, is likely to be saved if, as expected, the Dutch government accepts a rescue plan being submitted it next week.

A report from TNO, whose conclusions are expected to be approved by the end of the year, says that the centre should be turned into an independent foundation, with its running costs shared equally by the government, the commission of the European Union, and industry.

The centre already carries out a significant amount of work for the European Commission. But in 1992, facing severe budget cuts, the TNO decided it could no longer afford to run its national primate centre, which had run up a deficit of Dfl13.7 million (US\$2 million) (see *Nature* 363, 4; 1993).

The decision caused an outcry from scientists in the Netherlands and elsewhere, who emphasized the high standard of research at the centre (for example, its pioneering work on bone marrow transplantation) and the importance of its breeding colonies for AIDS research. This forced TNO to think again, and to press the government for an alternative solution.

If its rescue plan is approved, the centre — to be known as the Biomedical Primate Research Centre — will operate as an independent body. One third of its Dfl13.5 million annual operating costs would be guaranteed by the Dutch government for four years, with the difference made up with funds from the commission's fourth framework programme and contracts with pharmaceutical companies. **A. A.**

Genetics institute opens in Strasbourg

Paris. The French Institute of Genetics and Molecular Biology (right) officially opened last month at Illkirch-Graffenstaden near Strasbourg in France. In the first collaboration of its kind for French science, the centre was built with the support of a private company, Bristol-Myers Squibb. It has a floor area of 15,000 square metres.

The centre is directed by Pierre Chambon, whose research group was recently ranked by *The Scientist* as sixth in the world league of leading genetics research institutes, being credited with 30 per cent of French

publications in *Nature* and 40 per cent of those in *Cell*. The building was designed by the US architect K. Kornberg, the son of the Nobel prizewinner Arthur Kornberg.

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REASONS

Henri Parent

Australians protest limit on support for gravity research

Sydney. Australian scientists are disappointed at a decision by the Australian Research Council (ARC) to provide less than half the funding requested by a consortium of research groups involved in an international project to develop gravitational wave detectors.

Although the project grant awarded to the consortium — A\$1.25 million (US\$937,000) over three years — is the biggest ever made by the research council, it is still considerably less than the A\$3 million the consortium had asked for.

Mary O'Kane, the chair of the council's research grants committee, says that the decision reflects the pressure on the council in allocating its limited funds.

But John Sandeman, professor of physics at the Australian National University in Canberra, ACT, who coordinated the consortium that made the bid, points out that the grant contrasts sharply with the US\$250 million being put into the project by the US government and US\$100 million by a joint French-Italian contribution.

Sandeman says that the shortfall in the grant will mean cuts in research projects in which Australia is among the world leaders. Some of those involved in the grants process "did not understand" the nature of the application, he said.

The grant was made under ARC's large grants programme, which accounts for A\$83 million of the A\$200 million awarded annually by the council; the rest is distributed through a series of schemes, including support for special centres and fellowships.

Even so, the council, which is the largest source of funding for pure science in Australia, has been able to support only 22 per cent of the 3,004 applications for large grants.

O'Kane says that she sympathizes with Sandeman's disappointment, but argues that the only solution is for the government to increase the money available for the large grants programme to \$200 million.

According to Sandeman, the joint application was really six–seven large grant applications rolled into one, in order to support work by research groups at several universities. These include one headed by David Blair at the University of Western Australia in Perth, who is working on ways of isolating the proposed detectors from interference, and another at the University of Adelaide headed by Jesper Munch, which is developing the laser equipment required.

Sandeman said that the planned work, which is an essential part of the international project, will have to be pruned to meet the new budget restrictions. But he does not yet know which areas are likely to be cut back.

Mark Lawson

'Car of the future' project off to bumpy start, says panel

Washington. The Clinton administration's flagship technology programme to develop more fuel-efficient cars has been strongly criticized by a National Academy of Engineering panel for lacking either a proper programme plan or an adequate management structure a year after its launch.

Using uncommonly robust language in its first report on the 10-year Partnership for a New Generation of Vehicles (PNGV) programme, the panel says it is "disconcerting that the PNGV is essentially a year into the partnership and was unable to provide detailed and defined program plans, schedules and milestones".

The academy panel is chaired by Trevor Jones, a recently retired British motor industry executive. In a report published this week, it claims that the existing management structure of the programme is "too

But, as the most visible element of Clinton's technology policy, PNGV is being watched warily both by political opponents, who see it as an unnecessary government intervention in industry, and by some scientists, who fear that it will divert government money from basic research.

The report by the National Academy of Engineering panel says that the project management lacks coherence on both the government side, where many departments and agencies are involved, and on the industry side, which the three companies are operating through their existing research collaboration body, the United States Council for Automotive Research (USCAR).

It calls for a new programme management office to take charge on the government side and for the appointment of a technical director to lead an integrated project team on the industry side.

The report also warns of budget problems as the project gathers steam and tries to draw funding from existing programmes inside different government agencies. It calls for the entire PNGV budget to appear as a single-line item in the budget now under preparation for the 1996 financial year, which begins next October, under the control of a central programme management office.

The academy panel says that PNGV urgently needs to widen its base of support to include both the general public and those congressmen who have ultimate control over its budget. "Currently, the programme is almost exclusively a Clinton administration-USCAR initiative," it warns.

But the panel also says that it was encouraged by the good relationships developing between people from government and industry engaged in the programme, and by their enthusiasm. It also found that the goals of the programme — including the 80 m.p.g. car — were attainable, if the exercise is properly managed.

So far, the response of USCAR has been muted. "We are pleased that they have recognized the significant progress this project has made in its first year," says Scott Fosgard, a spokesman for the industry group. "We believe it offers valuable insight into our programme, and will consider its recommendations closely."

Before the report appeared, Mary Good, under-secretary for commerce and the administration official with overall responsibility for PNGV, said that it was going better than planned. "It started out slowly but over the last couple of months it has begun to gel," she said, adding that the review process in August had served as an opportunity "for people to get their acts together".

Colin MacLiwain



diffuse and its capability too limited" to attain PNGV's main goal, namely the development of a car with three times the fuel efficiency of existing models, equivalent to about 80 miles to the gallon.

PNGV was set up by the administration to involve the 'Big Three' US car makers — General Motors, Ford and Chrysler — in research and development collaboration with government agencies, led by the commerce and energy departments. It was expected to include about \$3 billion of government research funds diverted from other programmes over 10 years, to be matched by investment from the three car companies. Most of the industry contribution would come in the later stages of the programme.

The programme was launched in September 1993 by President Bill Clinton, Vice President Al Gore and the presidents of the three car companies, and the group met again at the White House last month to celebrate publicly the programme's first birthday.

UK under attack over lab 'efficiency' review

London. Britain's Royal Society has published a swingeing attack on outline proposals to reorganize 53 publicly funded research laboratories through a massive programme of 'rationalization'.

The proposals were the result of a three-month study of the laboratories by a group of government 'efficiency experts'. Their report, published in April, backed down from initial suggestions that many of the laboratories might be privatized; but it still suggested wide-ranging changes to avoid what the team described as duplication and overlap (see *Nature* 368, 681; 1994).

But the Royal Society, in one of the strongest criticisms it has ever made of the government, claims that the proposals are at best a misunderstanding of the role of the laboratories in providing a scientific underpinning to the nation's industrial activities, and at worst a dangerous exercise that could undermine the country's science base.

"The Scrutiny Review has not been able to produce coherent, well-judged recommendations," the society says in a statement published this week as its response to the government's request for comments on the review's proposals.

Pointing out that British science is already in the middle of upheavals resulting from last year's white paper on science, the society says that there is "no case for pro-

ceeding now with what it proposes, which would entail extensive and costly disruption for no obvious benefit".

The statement describes efforts to present the review purely as an exercise in management efficiency, divorced from questions about the mission of the laboratories it studied, as "disingenuous".

It claims that the team of civil servants that carried out the review was unable to give "even an approximate indication of the scale of savings that its proposals might achieve". Nor, it claims, was the team able to demonstrate "the existence of real scientific overlap on a scale which would warrant the disruption of major restructuring".

The Royal Society says that it is unenthusiastic about both models that the team suggests as a basis for restructuring. One would group research agencies according to their field of interest; the others would seek groupings based on regional proximity.

It also claims that the report fails to acknowledge the large institutional and social costs of restructuring, and that the review "illustrates the dangers of concentrating on financial issues without taking the trouble to obtain expert disinterested advice about the scientific and technological issues".

Sir Francis Graham-Smith, the director of the Nuffield Radio-astronomy Laboratories at Jodrell Bank, and physical sciences

secretary of the Royal Society, says that the review team "has not understood the scientific side of what they are saying, perhaps because they were initially sent in with heavy boots labelled 'privatization', even though they then backed off to concentrate on 'rationalization'".

The review team's proposals are thought to have come in for widespread criticism during the public consultation period on its recommendations, which ends tomorrow (11 November). The Institution of Professionals, Managers and Specialists, for example, the trade union that represents many of the scientists employed in the laboratories being assessed, said that its conclusions attempt to resolve genuine conflicts facing the laboratories "in favour of government market ideology, rather than the effectiveness of public science".

Addressing a House of Commons select committee two weeks ago, David Hunt, the minister for science, said that he supported the main goal of the efficiency review, namely to ensure that more money was spent on science and less on bureaucratic administration. But he also said that he had an open mind on the review group's proposals, and would not reach firm conclusions on future action before he had considered the various comments that had been made on its recommendation.

David Dickson

Lay panel backs gene-modified plants but urges stricter monitoring

London. Britain's first 'consensus conference', intended to canvas public opinion on plant biotechnology has resulted in calls for clear and meaningful labelling of genetically modified food products and a stricter system for monitoring genetically engineered crops.

These were the main recommendations of a group of 16 individuals, chosen from a wide range of ages and backgrounds as members of a 'lay panel', which last week published a preliminary report after two days of discussion and oral evidence from a range of biotechnology experts.

The experiment was funded by the Biotechnology and Biological Sciences Research Council. According to Tom Blundell, the council's chief executive, last week's conference, which was organized by the Science Museum on the council's behalf, cost more than £80,000, but it was "worth every penny".

The consensus conference was based on similar exercises elsewhere in Europe and in the United States. In Denmark in particular consensus conferences on topics such as food irradiation, mapping of the human genome and genetically manipulated animals have been used in public policy-making.

The British lay panel had undergone two weekends of intensive briefing on plant

biotechnology. Among the seven key questions they highlighted were: what are the principal risks and benefits of modern plant biotechnology; what impact could it have on the consumer and the environment; why is patenting so important; and what are the prospects for effective regulation?

Opening the conference, John Durant, assistant director of the Science Museum,



Long-life: Zeneca's modified tomatoes (left) after four weeks' storage.

said that the Danish experience with consensus conferences had shown that "it is an important enough process for us to try and see if it can play a role in the United Kingdom".

In its report the panel emphasized the potential benefits and risks of plant biotechnology. Benefits include plant varieties with higher yields, tastier and more nutritional fruits and vegetables, less use of herbicides and pesticides; but the panel also pointed

out the dangers of disruption to the food chain, the creation of new 'superweeds' and increased monoculture in agriculture.

One of its main recommendations centred on the right of consumers to choose whether or not to consume genetically modified food products. This would require "clear meaningful labelling", with national and international uniformity on procedures being the ultimate aim. The panel concluded that such labelling should be required by law, and not remain voluntary.

Several of the experts disagreed. Lord Howie of Troon, for example, chairman of a House of Lords select committee that recently complained of excessive regulation of the British biotechnology industry, said that labelling would signal that the products were dangerous. While welcoming the consensus method, he added "we must recognize that it doesn't have ultimate value".

The panel also felt that, despite its acknowledged stringency, there was "still room for improvement" in the United Kingdom's system of regulatory control of genetic engineering. In particular, it called for the appointment of an "independent ombudsman" to monitor investigations of the adequacy of the regulation, and for a government minister to oversee the development of monitoring procedures.

Maggie Verrall

Single amino-acid makes big difference in patent court

Tokyo. The US biotechnology company Genentech has suffered an unexpected defeat in Japan in a battle to defend its patent rights on the heart treatment drug tissue plasminogen activator (TPA).

The decision has highlighted the tendency of the Japanese system to place very narrow limits on the coverage of patents. The rejection of Genentech's claim that its patent had been infringed by Sumitomo Pharmaceutical hinged on a difference of only one in the partial amino-acid sequences of its own and the Japanese product.

The ruling was made on 27 October by a local district court judge in Osaka. The decision surprised everyone — including Sumitomo — as three years ago the US company won a very similar suit against another Japanese company, Toyobo, which was subsequently forced to halt its production of TPA (*Nature* 354, 4, 1991).

Genentech made worldwide patent applications in 1983 for its TPA, made with recombinant-DNA technology. But the Japanese patent office refused to accept Genentech's bid for a patent covering every form of TPA, including that which occurs naturally. Genentech had to revise its Japanese patent application several times by providing key amino-acid sequences before the patent was accepted in January 1991.

In the case of Toyobo's TPA, produced under a licence from the US company Genzyme Corporation, the amino-acid sequence was identical to that in Genentech's patent. But Sumitomo's TPA, made with technology licensed from the Wellcome Foundation in the United Kingdom, differs in one position of the amino-acid sequence.

The judge concluded that this was sufficient grounds for rejecting Genentech's claim of infringement.

The judge's decision has caused surprise largely because there does not appear to be any significant difference in the clinical effect of the two forms of the TPA; indeed the judge ruled that he could find no "meaningful" difference in clinical effect. Nevertheless, he still argued that it could not be said that the immunological characteristics and biological activity of the two forms of TPA are the same.

Genentech has not yet decided whether to appeal against the decision. The market for TPA in Japan is only about ¥6.5 billion (US\$65 million) a year and falling — many Japanese doctors do not use clot-dissolving agents such as TPA, preferring to use percutaneous transluminal coronary angioplasty, an invasive technique where a balloon is inflated to dilate the narrowed artery after insertion via a catheter.

But few would be surprised if Genentech does decide to appeal, as it has been championing the protection of intellectual property rights and pushing for reform of the Japanese patent system.

Patents tend to be very narrowly defined in Japan so companies can often circumvent patents held by competitors with only very slight modifications of a product or process.

Nevertheless, Sumitomo Pharmaceutical was still caught off balance by the decision, which had been widely expected to go in Genentech's favour. Sumitomo is now hurriedly producing promotional pamphlets and designing packaging for their product.

David Swinbanks

Eugenics law puts Beijing meeting at risk

London. The International Genetics Federation (IGF) may change its plans to hold the 1998 International Congress of Genetics in Beijing, following the Chinese government's decision last month to approve a law forbidding people with mental disabilities or contagious diseases to marry.

According to the official Xinhua news agency, the new law will "help reduce births of physically or mentally abnormal babies". The National People's Congress stated that it will also help to "upgrade the general qualities of the new population".

The law is a diluted version of a bill first proposed in December 1993 (see *Nature* 367, 3, 1994). Following widespread protests from the international scientific community, the idea seemed to have been dropped.

The IGF chose China to host the congress, held every five years, at its last con-

gress in Birmingham in 1993. But, concerned about the possible introduction of a 'eugenics' law, it subsequently obtained an agreement that, prior to the 1998 congress, the Chinese organizers would issue a statement reassuring the international community on the government's plans.

In a statement issued last weekend, the IGF says that the passing of the new law "has given the IGF new cause for concern", adding that it has initiated a "reappraisal" of China as an appropriate location for the 1998 congress.

The IGF has recently launched several initiatives designed to promote genetics at an international level. One is to take steps to increase the number of scientists from developing countries attending its congresses. Another is to bring pressure to bear on countries where there is potential for abuse of genetics.

Maggie Verrall

Former CNRS chief rejects criticism, hits at funding cuts

Paris. François Kourilsky, former director-general of the French Centre National de la Recherche Scientifique (CNRS), last week claimed that the government is misrepresenting the causes of the current financial crisis at the agency in order to cover up its decision to reduce funding for science.

Kourilsky was speaking after François Fillon, the minister of higher education and research, had blamed the crisis on past management of CNRS, which he described as "lax" and "calamitous". Kourilsky described the allegation as "absurd", and added that, if



Kourilsky: charges of laxity are 'absurd'.

the government wants to cut research funding, it should say so.

The crisis has arisen because of a decision by Guy Aubert, the agency's new director-general, that researchers can spend no more than 60 per cent of funds allocated for this year. The move was made to deal with a FF550-million (US\$106-million) gap in CNRS's finances caused by the government's failure to make promised payments.

Kourilsky argues that the state — not the CNRS — should make up the difference. In previous years he filled the gap by taking money from CNRS central funds and sees no reason why Aubert should not have done likewise. Indeed, following protests from researchers, Fillon last month authorized FF360 million to be taken from central funds.

Kourilsky also claims that the central question is the future of research in France. "Research policy cannot be reduced to making economies, and lack of financial support cannot be hidden behind allegations of past management," he says.

Researchers were due to hold a national strike earlier this week to protest about a reduction in support. Another concern is that the government may be intending to restructure CNRS and reduce the number of its laboratories from 1,300 to as few as 400, with the control of many passing directly to universities.

Observers say that Fillon is under pressure to propose reforms because Jacques Chirac, a conservative candidate for next year's presidential elections, wants to refer to research in his election manifesto — not least because Jacques Delors, the likely socialist candidate, will probably do so, having made research a major part of his recent white paper on European competitiveness (see *Nature* 366, 599; 1993). **Declan Butler**

Looking for drugs in ancient texts

SIR — We wholeheartedly agree with the interesting possibilities raised by Bart K. Holland (*Nature* 369, 702; 1994). A new look at ancient texts may certainly help us to discover new and more efficient drugs.

Holland mentioned various manuscripts written by Chinese and Greeks. He might also have mentioned texts written by Hindus in India. The sages of that era (1000–100 BC) had a clear concept of different diseases and their treatment; for example, they could differentiate between intermittent and continuous fevers and between diarrhoea and dysentery, using separate remedies for each. Medical texts such as the Caraka-Samihita and the Susruta-Samihita describe the use of more than a thousand plants in a wide range of diseases. They are available as Sanskrit and Hindi manuscripts and the remedies described in them are still used by practising Ayurvedic physicians in India (S. Gupta, *Ayurvedic System of Medicine* Vol. 1. N. N. Sen, Calcutta, 1909). The sad part is that Indians have lost faith in this system.

Interest has been rekindled in medicines from plants by the discovery of artemisinin from *Artemisia annua* for the treatment of malaria. A similar search of ancient Hindu texts would pay rich dividends in suggesting where to look for new pharmaceuticals.

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Floods explained

SIR — Your news story (*Nature* 369, 348; 1994) on the White House task force charged with framing a policy response to the Great Mississippi Flood of 1993 reveals that the focus of the draft version of their report is on floodplain and river management policies. Regrettably, it appears that the task force will ignore the broader landscape context, which is the profound human alteration of the natural hydrological cycle in the uplands.

The uplands of the Upper Mississippi and Missouri river watersheds originally consisted of native prairie, which has a high water-retention capacity. The native prairie has been completely replaced by intensive, industrialized, row-crop agriculture, resulting in a drastic reduction of

the upland watershed's water-retention role in the hydrological cycle. Current agricultural practices increase surface runoff rate directly through the systems of drainage tiles, drainage ditches and channelling of smaller streams. Indirectly, present agricultural practices increase surface runoff through soil compaction, which reduces infiltration, and through replacement of native perennial vegetation (prairie) by annual crops, which reduces transpiration. Both the direct and indirect increases in surface runoff, combined with increased erosional sedimentation, exacerbate the problems associated with the constriction of floodplains by levees and the loss of floodplain wetlands.

Recognition of the profound agricultural alteration of the original upland watershed of the Upper Mississippi and Missouri rivers is critical if we are fully to understand the cause of the Great Mississippi Flood of 1993 and the likelihood of future such floods.

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No demons

SIR — In his review of R. Steven Turner's book *In the Eye's Mind: Vision and the Helmholtz–Hering Controversy* (*Nature* 370, 259–260; 1994), C. R. Cavonius writes that: "It is unfortunate that an otherwise well-produced book has fallen into the clutches of that desktop publishing demon, the lost data-file: all bibliographic references to Helmholtz are missing. The publisher should try to make them available."

In fact, in the "Abbreviations" of the book's "Abbreviations and References" section, the author lists all of the necessary bibliographic references to Helmholtz's work.

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Counting time

SIR — Cesare Emiliani (*Nature* 366, 716; 1994) proposes a new calendar that would start in the Late Glacial at what he calls a Human Era, instead of at the birth of Christ. An interesting and commendable idea indeed, which could make life somewhat easier for the Holocene stratigraphers, but more complicated for historians. As a supporting argument, Emiliani claims that the interval from –1.5 (or 1.5 BC) to +1.5 (or 1.5 AD) cannot be computed by conventional algebraic methods because it covers only one and not three years.

I do not understand his mathematics. My understanding is that the BC/AD boundary is taken arbitrarily as the last midnight of the year in which Christ is believed to have been born on 25 December. If so, then 1.5 years AD means 18 months following 00 Z of 1 January of 1 AD, or the midnight of 1 July of 2 AD. Correspondingly, 1.5 years BC means 18 months before that same timeline, which turns out to be July of the year 2 BC. That makes 36 months or three years altogether.

Sometimes we even hear about an intercalary year zero, which would extend the interval by another year. In no instance, however, can we come up with a single year for the corresponding timespan. Unless the year zero is inserted in the system, there should thus be little difficulty in computing time across the BC/AD boundary by normal algebraic procedures.

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Masculine view

SIR — In a recent *Daedalus* feature (*Nature* 370, 332; 1994), it is implied that a male brain without testosterone would be the equivalent of the female brain. A humorous experiment is then suggested, where male subjects would take a fictional drug, Neutermind, that denies testosterone access to the brain. Given the earlier premise and later comments, we must assume that *Daedalus* believes this state of mind, rather than being neuter, would actually more closely resemble a "feminine mind". The article notes that Freud would predict that these men's scientific creativity would summarily fade, although *Daedalus* stopped just short of advocating this viewpoint. It was stated, however, that if male criminals were given the drug they would turn into "placid, unadventurous citizens".

Ironically, just 12 pages away, you report that the government of India is attempting to reduce the practice of gender-selective abortion and that 50,000–80,000 female fetuses are aborted every year after tests determine the gender of the fetus. In addition, you point out that female infanticide continues in India, where 6,000 baby girls have been murdered in one small community in Tamil Nadu over the past ten years.

If one of the world's preeminent scientific journals questions the value of women's minds, how does the government of India stand a chance of convincing its people of women's worth?

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Ranking Japan's life science research

Shigeaki Yamazaki

Some surprising facts about the most productive institutions emerge when Japanese life-science research is subject to a novel type of assessment.

THERE is growing international interest in the impartial external evaluation of research institutes and universities. Japan, although behind in this field, is trying to introduce such assessments¹. Citation impact data are often used for evaluation², but can easily be biased by a few highly cited papers³. I have evaluated Japan's medical research organizations based on the total output of papers, and the annual paper output per head, in international publications listed in the Medline database. My analysis reveals some interesting and perhaps unexpected rankings of Japanese medical research organizations, and this approach, which can easily be carried out using the Medline database on CD-ROM, should be generally applicable to medical research organizations in other countries.

A search of the Medline database for the first half of 1993 reveals that some Japanese universities are producing a comparable number of papers to some of the leading universities in the United States and Europe (Table 1), despite the fact that my analysis includes only English-language publications and does not include many papers published by Japanese researchers in Japanese. In terms of paper output per head, some Japanese universities also compare favourably with the United States and Europe, but Tokyo University, considered to be Japan's most prestigious university, has less than half the output per head of Kyushu University.

Although the Japanese medical research community publishes a huge number of papers in Japanese — in 1992 109,700 papers from 2,250 domestic journals were indexed in *Japana Centra Revue Medica* — there is a strong tendency for Japanese medical researchers to submit their best papers, particularly those in basic, rather than clinical, research, to international journals. In 1992, Japanese researchers in the life sciences published 15,000 papers in international journals and 6,000 papers in English-language Japanese journals indexed in Medline.

Survey

I analysed 7,607 Japanese papers published in international journals from Medline on CD-ROM for the first half of 1993. I did not include English-language Japanese journals as some may be of inferior quality to international journals. I chose Medline for this survey because it

has a broad international coverage. The highest quality and most useful 3,700 journal titles that rely on the peer-review system are selected for the Medline database irrespective of their place of publication. Medline is published by the National Library of Medicine (NLM) in the United States and is the main database for secondary reference material in the life sciences. NLM receives more than 20,000 international life-science journals, of which 3,700 are selected for Medline to provide a database of key biology journals. I used the number of publications in journals contained in Medline as a basis for the output of an institution. I used two criteria: the total number of papers from each institution and the annual paper output per head.

In determining the number of researchers at each institution, I used the *Directory of Medical Education Organizations 1992-1993* (published by Yodosha Co., Tokyo) for the faculty of medicine at universities and the *Directory of Medical Science Researchers 1992-1993* (also published by Yodosha) for the faculty of pharmaceutical sciences, the faculty of dentistry, and the national and private research institutes. These directories do not include research assistants and graduate students, who are sometimes among the most prolific paper-writers and thus really need to be included. Using the *Nationwide List of Research Institutes 93-94* compiled by the Science and Technology Agency in Japan, I was able to obtain the total number of researchers, including research assistants and graduate students.

Universities

The three national medical schools in Osaka, Kyoto and Kyushu come out with

high rankings in the number of papers (Table 2). In terms of the paper output per head, Kyushu University takes first place, and its rank is outstanding. Its medical scientists are encouraged to publish papers, especially in English⁴. Although the University of Tokyo is Japan's most famous university, its medical school, judged from the paper output per head, is ranked lower. A comparison between the national medical schools and private medical schools shows that the former have much higher productivities, largely because the latter are far less active, and emphasize clinical services rather than research. Generally speaking, in Japan, the reputation of national universities is higher than that of private universities, which is the opposite situation to that in some Western countries.

The top 15 medical schools and 5 pharmaceutical-sciences schools are listed in Table 2 (see next page) according to three kinds of ranking: number of papers; output per head based on the number per faculty (professors, associate professors and lecturers); and paper output per head based on the total number of researchers (including research assistants and graduate students). Among the national universities (Tokyo, Kyoto, Osaka, Tohoku, Kyushu, Hokkaido and Nagoya) rank highest.

Large-sized national universities, especially the former imperial universities, have many research assistants and graduate students. Comparing the two kinds of paper output per head, most of the large-sized national universities tend to rank lower when research assistants and graduate students are included, whereas small and medium-sized national universities with high productivity, for example Shi-

TABLE 1 LEADING MEDICAL SCHOOLS IN THE UNITED STATES, UNITED KINGDOM AND JAPAN

Medical School	No. of papers	Researchers	Per head (annual)
Johns Hopkins	538	3,411	0.32
Columbia	386	2,127	0.36
Cornell	304	1,650	0.37
Oxford	246	489	1.01
Osaka	244	884	0.55
Kyushu	226	480	0.94
Tokyo	218	1,051	0.41

The number of papers was taken from the Medline database for the first half of 1993. Researchers are defined as people officially associated with the medical school as full-time teachers regardless of faculty rank, including postdoctoral fellows, research assistants and graduate students. The number of researchers was obtained directly from each medical school. Though Japanese researchers, in general, have a language barrier, they publish English papers in prestigious international journals. The productivity of major institutions in Japan is equal to that of leading universities in the United States and Europe.

mane, Gifu, Gunma and Shinshu, tend to rank higher.

The average annual paper output per head based on the total number of researchers in the top 15 medical schools is 0.55. The analysis shows that researchers in Japan's top medical schools produce on average one paper every 2 years in international journals.

This result is different from a report ranking Japanese universities by the citation impact rate (average number of citations per paper) in biological sciences between 1981 and 1991 in which Osaka University was ranked first, Kyoto University second, and Tokyo University third². I have ranked Tokyo lower and Kyushu much higher. Although there has been an increasing use of citation data for measuring scientific performance, ranking a university solely by its citation impact can be misleading, because one or two highly cited papers can raise the rate substantially³.

Turning to national medical schools other than the seven former imperial universities, Kumamoto and Kobe ranked highest for paper output per head based on the number per faculty. These medical schools contain some bright research stars. Although the private medical schools have many faculty members, fewer papers are produced. Keio University School of Medicine, a leading private university, is ranked twelfth in paper output per head based on the total number of researchers. Compared with the high productivity of the schools of medicine and pharmaceutical sciences, the paper production at dental schools is low and research performance is poor.

Research institutes

The ranking of research institutes by number of papers shows the National Cardiovascular Center takes first place and the National Cancer Center Research Institute comes second (Table 3). Both are key research institutes for conducting clinical investigations and providing advanced treatment. The Institute of Physical and Chemical Research (RIKEN) takes third place, and the Mitsubishi Kasei Institute of Life Sciences is fourth. RIKEN has about 500 researchers and engineers as permanent staff and is rapidly becoming an international centre of excellence in Japan. The output per head of this institute does not rank so high in my analysis because many of RIKEN's researchers are not involved in life-science research.

In terms of the paper output per head, the National Cardiovascular Center holds first place and the Mitsubishi Kasei Institute of Life Sciences is second. This institute was established in 1971 as a private foundation, and is now one of Japan's leading life-science research institutes. The Protein Engineering Research Insti-

TABLE 2 RANKINGS OF UNIVERSITIES

Medical schools			Output of faculty			
Rank	Number of papers		Output of faculty		Output of faculty, res. assist, grad. stud.	
	Name	Paper	Name	Per head (annual)	Name	Per head (annual)
1	*Osaka	244	*Kyushu	2.42	*Kyushu	0.94
2	*Kyoto	233	*Osaka	1.95	Shimane	0.75
3	*Kyushu	226	*Kyoto	1.89	*Tohoku	0.66
4	*Tokyo	218	*Nagoya	1.67	*Osaka	0.55
5	*Tohoku	172	*Tohoku	1.54	Gifu	0.55
6	*Nagoya	138	Kumamoto	1.36	Gunma	0.53
7	†Keio	130	Kobe	1.34	Shinshu	0.50
8	Tokyo Med. & Dent.	102	Shimane	1.33	*Kyoto	0.50
9	Kumamoto	96	†Keio	1.31	*Nagoya	0.47
10	Kanazawa	91	Gifu	1.19	Kyoto Prefectural	0.47
11	†Kansai Med.	89	Shinshu	1.17	Niigata	0.46
12	Kyoto Prefectural	84	Osaka City	1.16	†Keio	0.46
13	*Hokkaido	83	*Tokyo	1.14	Kumamoto	0.45
14	Kobe	78	Kanazawa	1.06	*Tokyo	0.41
15	Niigata	77	Kyoto Prefectural	1.04	Tokyo Med. & Dent.	0.40
Pharmaceutical schools						
Rank	Name	Paper	Name	Per head (annual)	Name	Per head (annual)
1	*Tokyo	35	*Tokyo	2.33	Okayama	1.25
2	*Osaka	27	*Osaka	1.93	*Osaka	0.64
3	Okayama	25	Okayama	1.92	*Kyushu	0.48
4	*Kyoto	24	*Kyoto	1.66	*Kyoto	0.47
5	*Kyushu	19	*Kyushu	1.52	Gifu Pharm. U.	0.46

*:Former imperial university; †:private university.

Three kinds of ranking are shown. The first is based on the number of papers. The second is the paper output per head based on the number of faculty consisting of professors, associate professors, and lecturers. The third is the paper output per head based on the total number of researchers including postdoctoral fellows, research assistants and graduate students.

TABLE 3 RANKING OF RESEARCH INSTITUTES

Name	Papers	Rank	per head (annual)	Name
National Cardiovascular Ctr	70	1	0.92	National Cardiovascular Ctr
National Cancer Center Res. Inst.	42	2	0.68	Mitsubishi Kasei Inst. Life Sci.
Inst. Physical & Chemical Res. (RIKEN)	40	3	0.68	National Cancer Center Res. Inst.
Mitsubishi Kasei Inst. Life Sci.	32	4	0.66	Protein Engineering Res. Inst.
Tokyo Metropolitan Inst. Gerontology	29	5	0.50	National Inst. Genetics
National Inst. Hygienic Sciences	27	6	0.50	Tokyo Metropolitan Inst. Neuroscience
Ctr Adult Diseases	26	7	0.48	Osaka Bioscience Inst.
National Inst. Health	20	8	0.48	Tokyo Inst. Psychiatry
National Inst. Physiol. Sci. Okazaki	20	9	0.46	Aichi Prefectural Colony
Cancer Institute	18	10	0.38	National Institute Neuroscience

tute ranked fourth, and the Osaka Bioscience Institute seventh: both of these are examples of newly established semiprivate research institutes⁵. At the Osaka Bioscience Institute, founded in 1987, peer-review committees evaluate the output of scientists to strengthen the institute's research. The National Institute of Genetics ranks fifth, reflecting its active research role. Private or semiprivate research institutes appear to be more productive on a per capita basis than national ones. Most of the large-sized national basic research institutes are low in rank in terms of paper output per head.

This survey shows that the paper output per head can provide a measure for evaluating scientific activity in Japan. Evaluation of research performance is coming to be seen as an integral part of science.

Bibliometric evaluation provides unbiased judgment, not by an inner circle of peers but by neutral outside observers. The scientific community of Japan, as well as that elsewhere in the world, cannot avoid such evaluations. □

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The return of thermonuclear fusion

Four research reports from Lawrence Livermore Laboratory amply justify the decision to build a more powerful laser for fusion research.

WHATEVER happened to thermonuclear fusion? Much has changed since 1957, when Britain's Atomic Energy Authority (as it then was) made public its belief that a simple toroidal machine called ZETA had already demonstrated the fusion of hydrogen and deuterium nuclei on a small scale. The most poignant proof of the unsustainable optimism at Harwell (where the machine had been built) was the two-foot-thick concrete shield intended to protect the staff from the expected neutron flux. ZETA did indeed generate neutrons.

Optimism has not, of course, been banished by that unfortunate experience. There are a dozen significant laboratories around the world at which the torch is kept alight — at Princeton (New Jersey), Munich (Bavaria), St Petersburg (Russia), Osaka (Japan) as well as at the collaborative European research establishment at Culham, only a few miles in Britain from where ZETA used to be. But the effect of the disappointments of 1957 is lasting. Even the enthusiasts for thermonuclear fusion are now exceedingly cautious in what they claim on behalf of their experiments.

Caution is certainly the keynote of the four papers, from the Lawrence Livermore Laboratory in California, which appear consecutively in *Physical Review Letters* for 24 October. Between them, they constitute the most complete account so far of the use that Livermore has been making of its huge Nova laser as a means of triggering the fusion of tiny globules of thermonuclear fuel enclosed in thin glass envelopes. The case for taking these papers seriously is that the US Department of Energy has only recently agreed that the Nova laser should be rebuilt, and given more energy. When the paymasters believe it worth going an extra mile, the rest of us must take some notice.

Nova's claim on public attention is, of course, its power output. A single pulse of radiation (at 350 nm, at the blue end of the visible spectrum) lasting only two nanoseconds may carry more than 20 kJ of energy. The public relations people are forever saying that this is the equivalent of 10 million megawatts in continuous operation (or a substantial fraction of the world's electricity generating capacity).

How can such a machine trigger thermonuclear fusion? Somehow it must be arranged that a globule filled with thermonuclear fuel is rapidly compressed. Shining laser light directly on such a globule would be unproductive; the target would simply evaporate. So the trick is to coat the globules

with an inert material capable of absorbing radiation and compressing the fuel by implosion. M.D. Cable and 13 Livermore colleagues describe one of the recipes for the design of a globule that appears to work well (73, 2316–2319; 1994). Capsules of glass 90 μm in radius with walls no more than 5 μm thick can apparently contain thermonuclear fuel gas (hydrogen, deuterium, tritium) at up to 200 atmospheres pressure. A 37 μm -thick coating of polymer then serves as the inward-driven implosion engine. Although the authors refer at some point to the "production line" on which the capsules are made, clearly it cannot be a mass-production line.

The complications are not yet over. Such dreams as there may originally have been that it would be possible to obtain thermonuclear fusion by dumping the energy of a laser pulse into a bare fuel globule have melted away. Livermore's essential innovation is the design of containers for the fuel capsules that are themselves wonders of precision engineering. Typically, the containers are little cylindrical boxes just 2.5 mm long and 1.6 mm in diameter, fashioned from uranium (for its inertial mass). In each of the circular ends of the box there is a hole, perhaps 1.2 mm in diameter. The geometry of the laser is arranged in such a way that Nova's total output is divided among 10 equal beams, five of which enter obliquely through each end-hole, irradiating the internal walls of the cylindrical box.

The result is that a laser pulse will turn the internal walls of the cylinder into a source of X-rays, evaporating material from the surface at the same time. That is the source of the pressure for driving the implosion of the thermonuclear fuel within each capsule. As well as uranium, the Nova group has used gold for a constructional material, and has tried out a variety of inner coatings for the cylindrical walls, nickel metal for example. They do not, unfortunately, explain how they get the globule into the box.

How well does this arrangement work? Remarkably, the Nova group reports density compression of the thermonuclear fuel by factors of 24 that is, at the same time, sufficiently uniform for the fuel globule to remain spherical. The temperature within the cavity, measured in electron-volts, increases with the duration of the laser pulse to 200 eV or so, well in excess of 10^6 K. And there are neutrons, the magical diagnostic of thermonuclear research.

Depending on the fuel mix, the primary neutron yield from a laser pulse appears to range from 10 to 100 million (with pure

deuterium fuel) and from 100 to 1,000 million (for a 50:50 mixture of deuterium and tritium). Interestingly, even with pure deuterium, where the first thermonuclear reaction is the formation of tritium (by $\text{D} + \text{D} \rightarrow \text{T} + \text{p}$), enough time elapses for the production of neutrons by the secondary reaction of tritium with deuterium (by $\text{D} + \text{T} \rightarrow {}^4\text{He} + \text{n}$). The neutron energy, at 14 MeV, is so much greater than that of other confounding signals that it becomes a telling diagnostic of what is happening in the compressed thermonuclear fuel.

The three other papers in this group of four deal with the details of this technology, a bizarre blend of the very big (a 20 kJ laser) and the very small (fuel capsules less than 0.25 mm in diameter). Following the course of the implosion has entailed making holes in the cylindrical cavity walls for the direct measurement of X-rays, for example (Kauffman, R. L. *et al.* 73, 2320–2323; 1994). And the quality of the implosion of the thermonuclear fuel turns out to depend sensitively on the roughness of the surfaces of the multi-layered fuel globule (Dittrich, T. R. *et al.* 73, 2324–2327; 1994).

The weight of Livermore's case, however, rests on the last of the four papers (Suter, L. J. *et al.* 73, 2328–2331; 1994), which includes a sprinkling of authors from Los Alamos, Livermore's *alter ego*. Among other things, for example, this group says that it has enough experience of laser-energized cavities to be able to adjust the points of impact of the ten beams on the internal cavity-walls to accommodate changes in the time-course of the driving pulse.

What does this imply for the feasibility of thermonuclear fusion by the inertial confinement route? Briefly, that there is still a long way to go. Putting delicately machined globules of fuel into even more carefully constructed cavities is unlikely to be an industrial technology in the near future. But the inertial confinement community hopes that the delicate capsules will be used as ignition devices for thermonuclear fuel compressed in some other way.

As in the parallel development of toroidal machines, the immediate need is to have a system in which the operating parameters are more nearly those that will have to be obtained in a real-life thermonuclear reactor. People will then be able to start planning the machines that may eventually produce usable power economically. It will be time enough to take up that question when the millennium has come and gone.

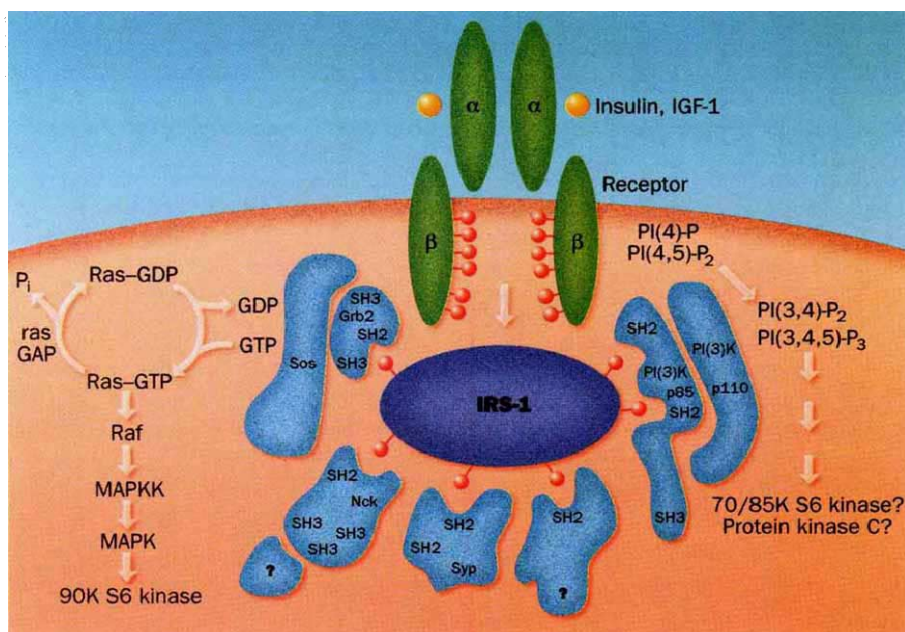
John Maddox

Life without the IRS

Gustav E. Lienhard

ONCE again the outcome of a gene knock-out experiment is both surprising and revealing. This time the victim is the gene encoding insulin receptor substrate-1 (IRS-1), which is thought to occupy a central place in the signalling pathways emanating from the receptors for insulin and insulin-like growth factor-1 (IGF-1), and the consequences are described by Tamemoto *et al.*¹ and Araki *et al.*² on pages 182 and 186 of this issue. Character-

proteins. So far, IRS-1 has been found to associate in this way with a lipid kinase (phosphatidylinositol-3-OH kinase, PI(3)K), a complex that catalyses the release of GDP from Ras (Grb2-Sos), a phosphotyrosine phosphatase (Syp), and a linker protein (Nck), the function of which is not yet clear. Binding to IRS-1 stimulates PI(3)K, Syp and, most probably, Grb2-Sos. The stimulation of these in turn triggers pathways leading to the



Insulin and IGF-1 signalling through IRS-1 (see the description in the text). In addition to the SH2 domain, several of the proteins contain one or more SH3 domains. PIP, phosphatidylinositol phosphate(s), with positions of ring substituents indicated in parentheses; rasGAP, GTPase-activating protein acting on Ras; the red balls are phosphates. See text for other abbreviations. (Adapted from ref. 14.)

ization of mice homozygous for the disrupted gene shows that IRS-1 is required for normal growth and glucose homeostasis, but also that much of the signalling by the insulin and IGF-1 receptors can be independent of IRS-1.

Shortly after the receptor for insulin and the homologous one for IGF-1 were found to be ligand-activated tyrosine kinases, the search for substrates turned up a relatively abundant phosphotyrosine-containing protein of relative molecular mass 170,000 (M_r 170K) with widespread tissue distribution. Studies on this protein (IRS-1) over the past three years have led to the concept that its role is to link these two receptors to a group of signalling proteins containing Src-homology 2 (SH2) domains (see figure)^{3,4}. The activated receptors phosphorylate IRS-1 on at least five tyrosine residues. Specific phosphotyrosine-containing sequences on IRS-1 then serve as binding sites for SH2 domains on particular signalling

activation of several protein kinases: in the case of PI(3)K, possibly the 70K/85K ribosomal S6 kinase⁵ and specific isotypes of protein kinase C; in the case of Grb2-Sos and possibly Syp⁶, the kinase cascade consisting of Raf, the mitogen-activated protein kinase kinase (MAPKK), MAP kinase itself, and the 90K ribosomal S6 kinase.

As several signalling pathways therefore radiate from IRS-1, the question arises as to how critical IRS-1 is for insulin and IGF-1 action. If all signalling from the insulin and IGF-1 receptors were to proceed through IRS-1, then abnormalities in *IRS-1* knockout mice should be at least as severe as those in mice lacking either the insulin or the IGF-1 receptor. Mice homozygous for a disrupted IGF-1-receptor gene are markedly retarded in their growth *in utero* (45 per cent of normal size), die at birth of respiratory failure, and have abnormal morphology of the central nervous system and skin⁷.

Rather surprisingly, mice homozygous for the deleted insulin receptor gene, or for the insulin gene itself, have not yet been reported, although a single case of a human infant lacking the insulin receptor gene has been described⁸. This child was an example of 'leprechaun' syndrome, characterized by "severe intrauterine growth retardation, marked hirsutism, wasted extremities, prominent abdomen and small triangular face with prominent eyes". Consequently, signalling through both the IGF-1 and insulin receptors is essential for normal intrauterine growth and development. This signalling is, of course, also critical for glucose homeostasis. Untreated insulin-dependent diabetes mellitus in humans and in the closely related model of this disease, the NOD mouse⁹, in which the insulin-producing pancreatic β -cells are destroyed by immune action, results in hyperglycaemia, ketoacidosis, weight loss and death.

In contrast to these strikingly severe symptoms, the phenotype of mice lacking IRS-1 is surprisingly mild^{1,2}. The mice suffer some retardation of intrauterine growth, their weight at and after birth being about 60 per cent of normal, but they have no apparent morphological abnormalities. Their blood glucose level after an overnight fast or an oral glucose bolus is normal, although an injection of insulin lowers their blood glucose only half as effectively as it does in wild-type mice. This blunt response is due, at least in part, to a reduction in the enhancement of glucose transport by insulin. Both studies^{1,2} report that insulin stimulation of glucose transport into isolated adipocytes from the mutant mice is only about 40 per cent of what it is in the wild type.

The conclusion from all this, then, is that much of the critical signalling from the insulin and IGF-1 receptors does not require IRS-1. With regard to the Ras-MAP kinase pathway, Tamemoto *et al.*¹ find that insulin activates MAP kinase as well in the livers of mutant mice as in those of normal mice. The basis for this effect is likely to be the tyrosine phosphorylation of another substrate protein, known as Shc, by the insulin receptor. The phosphotyrosine form of Shc, like IRS-1, binds to Grb2-Sos to activate the Ras-MAP kinase pathway. This route of activation probably functions whether IRS-1 is present or not, because insulin causes a comparable degree of tyrosine phosphorylation of Shc in the livers of normal and mutant mice. The implication is that the association of Grb2-Sos with IRS-1 usually contributes little to the MAP kinase pathway; indeed, studies with cultured cells have reached just this conclusion¹⁰⁻¹².

Another surprise from these studies came upon examination of PI(3)K activity. In normal cells, insulin causes a rapid increase in the level of phos-

phatidylinositol-3,4,5-triphosphate, and much evidence attributes this to the activation of PI(3)K as a result of its association with the phosphotyrosine form of IRS-1 (ref. 13). Consequently, the expectation was that, in contrast to the result with normal mice, little or no PI(3)K activity would be immunoprecipitated from the tissues of insulin-treated mutant mice by antibodies against phosphotyrosine. However, both groups^{1,2} found a considerable amount of PI(3)K activity in this immunoprecipitate from the liver after insulin administration (more than 50% of the value for the wild type), and Araki *et al.*² found the same in muscle. The reason for this unexpected result is the association of another insulin-elicited phosphotyrosine protein, of relative molecular mass about 180K, with PI(3)K. Using phosphotyrosine immunoblotting, Araki *et al.* discovered this protein in immunoprecipitates obtained from the liver and muscle of insulin-treated mutant mice with antibodies against phosphotyrosine or PI(3)K. Deletion of *IRS-1* has thus revealed a putative second substrate for the insulin receptor, dubbed IRS-2 by Araki *et al.*, which binds to and probably activates PI(3)K.

The inference that, even in the absence of IRS-1, insulin activates PI(3)K to some degree may account for the persistence of insulin-stimulated glucose transport in the mutant mice, albeit reduced. This latter effect is due to the translocation of additional glucose transporters (Glut4 isotype) to the plasma membrane, and studies with inhibitors of PI(3)K suggest that activation of the enzyme is necessary for this to happen^{5,14}.

Future research in this area will be aimed at cloning and characterizing IRS-2, this new player on the insulin signalling scene. It will surely include another knockout. □

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Weakening under stress

David J. Stevenson

WHEN a volcano on land erupts, or when new crust is made on the sea floor, the first step, tens of kilometres below, involves partial melting of rock and percolation of melt upwards. So Earth scientists are keen to find out how melt is distributed at the microscopic level and how this might affect the strength of the medium. On page 164 of this issue¹, Zhen-Ming Jin and colleagues report laboratory evidence for marked changes in melt distribution when they deform a partially molten 'mantle' rock, and an associated dramatic weakening of the rock. If the experiment is relevant to mantle-scale processes, it suggests that melt can be extracted more easily wherever deformation is going on — say, beneath mid-ocean ridges and possibly other volcanic centres.

Gravity dominates large-scale processes within the Earth and other planets; hot things rise because they are less dense and can lower the total gravitational energy by displacing denser material downwards. This is the ultimate cause of plate tectonics, which is driven by thermal expansion and contraction, and volcanism, driven by the density difference between liquid and solid, including the compositional changes that accompany this melting in a mineral mixture. However, volcanism generally requires only partial melting of the rock and the separation of melt from the mostly solid mixture. This separation requires melt percolation, which in turn depends on the melt distribution at the scale of individual rock grains.

This scale is in the realm of surface tension, the same process that determines the size of raindrops. It has been common practice, fairly well supported by experiment, to suppose that the upper mantle is a relatively simple system in which small amounts of melt distribute so as to form an interconnected framework of tubules along the triple junctions where three olivine grains meet. This simple topology suggests that melt can percolate rather readily (it will not be trapped) and the strength of the entire medium is not greatly changed from a solid with the same grain size. Over the past 15 years, modelling efforts based on these assumptions (for example, refs 2–5) have gone a long way towards providing a physical basis for explaining observations.

In recent years, though, problems have arisen at all length scales of interest. At the microscopic (grain) scale, some experimental work⁶ suggests that the distribution of melt is not as simple as previously supposed, with much of the melt distributed in plate-like structures between grain surfaces rather than at triple junctions. At the scale of centi-

metres to metres, there is concern about the extent to which melt equilibrates chemically with the solid through which it migrates. The evidence suggests only limited equilibration, perhaps because the melt migrates only a short distance by percolation before finding its way into broader channels where it can move rapidly upwards^{7,8}. At the grandest scale, we understand only poorly why oceanic crust is produced in a thin rift zone whereas the melting responsible for the crust is spread over many tens of kilometres to the flanks of the ridge.

The new experiments¹ may be relevant to all these issues. Jin and colleagues find a melt geometry that is dominated by tubules when the equilibrated partial melt is undeformed, but once the sample is stretched a little, the melt redistributes, spreading to grain boundaries. This behaviour has been previously observed for salts deforming in the presence of small amounts of intergranular brine. Apparently, this melt redistribution greatly aids grain boundary motion and greatly reduces strength. Presumably, it lowers the permeability for melt migration (which depends on the square of the width of a melt channel and is thus highest for roughly cylindrical channels). Nonetheless, the reduced strength of the matrix may aid the escape of melt, particularly if this process is accomplished at the scale of metres, as some geochemical data imply.

The experiments suggest an effective viscosity of around 10^{12} Pa s (the ratio of the stress, about 10^7 Pa, to the strain rate, about 10^{-5} s⁻¹). This value is some six orders of magnitude lower than commonly assumed for the Earth's asthenosphere. Although the experiments use stresses that are similar to those believed to develop in shallow subsolidus mantle, they are surely much greater than those that could develop in an extended region of partial melting.

One could, however, imagine a partial melt channel with locally high strain rate and an ability to focus upwelling material. For example, a channel only about a hundred metres wide could supply the entire mass flux that is believed to be

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appropriate beneath a mid-ocean ridge (around $10^{-4} \text{ m}^3 \text{ s}^{-1}$ per metre of mid-ocean ridge) if the material in the channel had a viscosity and inferred buoyancy like that observed in the experiments. This example, although extreme, is in the spirit of previously proposed explanations for the focusing of crustal formation at mid-ocean ridges^{4,9}.

But the biggest concern is the problem of scaling to mantle systems. The experimentally observed behaviour of the melt during deformation evidently disagrees with surface-energy minimization, suggesting that kinetics are important. But if kinetics (the speed with which melt

can freeze or new melt can form at interfaces) are important, then how can we translate experiments that last only tens of hours into mantle conditions over tens of thousands to millions of years? Unless we understand how to do this, we are not entitled to assume that the experiments tell us what happens in the mantle. That is the challenge for the future. □

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PALAEONTOLOGY

Shattering egg shells

Nicholas C. Fraser

ONE of the staples of displays and popular books about dinosaurs will have to change. This is the picture of a baby ceratopian dinosaur *Protoceratops* hatching from its egg, with caring parents hovering in the background. The problem is that the scene depicts the wrong parents. As described in last week's *Science*¹ by a team from the American Museum of Natural History and the Mongolian Academy of Sciences, an exquisitely preserved, mature embryo within one of these highly characteristic eggs is not a *Protoceratops* but a theropod dinosaur from the family Oviraptoridae — by definition one of the 'egg robbers' supposed to have dined on *Protoceratops* nests.

Ironically, it was discoveries by another American Museum field crew some 70 years earlier that resulted in the misconception. In 1922 the museum mounted an expedition, led by Roy Chapman Andrews, into Mongolia. The group's principal objective was to find mammal fossils, but the Gobi desert yielded incredible evidence of Upper Cretaceous dinosaurs (about 80–70 million years old), including both theropods and ornithischians. The jewels in the haul were undoubtedly the skeletons of the small ceratopian dinosaur *Protoceratops* and their putative eggs and nests. It was not unreasonable to suppose that the eggs were those of *Protoceratops* given the great abundance of protoceratopians, represented by various growth stages, and the extreme scarcity of theropods (the original collections from the Djadokhta Formation included 101 protoceratopians, but only four theropods in total, and only one oviraptorid). Over the years these finds have shaped many an exhibit and lecture.

The new material paints a very different picture. Moreover, to add a further gloss to the story, two skulls of very young

(probably embryonic) dromaeosaurid theropods were found with the nest. (Dromaeosaurs were active predators, popularized as 'raptors' in *Jurassic Park*.) Once again the reason for the association is not immediately apparent. Given the relatively undisturbed nature of the whole nest it seems unlikely that the skulls arrived there by chance. Perhaps the dromaeosaurids also hatched in the nest; if so, this would be the first evidence to suggest that some dinosaurs had adopted the parasitic nest habit that is typified by cuckoos. Alternatively, their presence could have been the result of the parent oviraptorids ransacking a neighbouring dromaeosaur nest.

Although dinosaur eggs were known before the 1920s, their occurrence had not been widely reported upon. Since then a number of such nests have been recovered from the Gobi desert, and with the discovery around the world of a number of other locations containing dinosaur eggs, research into this aspect of dinosaur palaeobiology has reached new heights². The classification of fossil eggshell has now become quite refined, and is typically based upon a number of fairly well defined characteristics. Shell macromorphology (shape, size, surface sculpturing and thickness) and histostructural pattern (type of pore canal system, arrangement of different microlayers), and ethological characters (pattern of arrangement of eggs in the nest), are very precise and when used together define very specific egg types. There can be no question that the eggs described by Norell *et al.*¹ are of exactly the same type as the numerous examples previously referred to protoceratopians. Their microstructure, with the angusticanaliculate pore system, is similar to that of ratite birds.

It now seems rather unfortunate that Henry Osborn gave *Oviraptor philocera-*

tops ('egg thief with a desire for ceratopian eggs') that name; he did so because the first specimen was found with its head crushed against a nest of the supposed *Protoceratops* eggs³. At the time it was postulated that a parent *Protoceratops* might have surprised the would-be thief, and exacted its revenge. In view of our current information this example of *Oviraptor* may have been protecting or even incubating the eggs when it died, and we could add that a more suitable name might have been '*Oviraptor philodromaeosaur*'. Alternatively this particular animal may have met its end plundering another oviraptorid nest.

Whatever the cause of death of this individual, there is no doubt that its short toothless jaws would have been efficient tools for breaking eggs (although some workers have suggested that oviraptorids could just have easily been herbivores, drawing a parallel with the herbivorous dicynodonts of the Permian). But in the light of the latest evidence, the question of just why *Protoceratops* is found with the oviraptorid eggs must now be asked — is there some palaeoecological information that we have failed to spot, or is the association perhaps just sheer chance?

The new discovery serves to emphasize the point that taxonomic assignment of eggs can be certain only when they are found with intact embryos and hatchlings, as for instance in the case of the hadrosaur *Maiaasauria* and the hypsilophodont *Orodromeus*^{4,5}. The lesson of caution is one that has recently been well taught in the circles of vertebrate palaeontologists. For example the rich beds of the Chañares Formation of Argentina⁶ have produced a wonderful diversity of Triassic archosaurs; but in many cases skeletal elements are closely associated rather than completely articulated, and it has emerged that some taxa are in fact chimaeras of two separate forms. The smoking gun might be pointing in the wrong direction — we also need the ballistics report on the bullet to confirm our suspicions.

When we add the finds of Norell *et al.*¹ to the discovery in 1992, also in the Djadokhta (and equivalent formations), of the curious flightless bird *Mononychus*⁷, we have to ask what other treasures await us in Mongolia. We are in a golden age of dinosaur research, and I suspect that the best is yet to come. □

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EARTH HISTORY

Strange bedfellows

Donald J. DePaolo

How long ago did the Earth start to form? Did the Moon form from the Earth? A report by Malcolm McCulloch in *Earth and Planetary Science Letters*¹ adds a new twist to both questions.

There are hardly two more fundamental questions that one could ask in Earth science, but convincing answers remain elusive. The difficulty in establishing the exact age of the Earth stems from the fact there is no geological record for the time between the Earth's origin, presumably about 4.5 billion years ago, and the time represented by the oldest Earth rocks, which are 3.8 billion years old. Ironically, the hardest evidence about the exact age of the Earth may come from lunar rocks, the oldest of which is firmly dated at 4.44 billion years². Now, the age of Moon rocks could conceivably have little to do with the age of the Earth, except that the preferred theory for the origin of the Moon is that it was produced from a giant meteorite impact with the Earth³. If the impact theory is accepted, then the Earth must have been around when the Moon formed, and its age must be bracketed by the age of the oldest Moon rocks and the age of the oldest meteorites, 4.56 billion years (see below).

ocean floor basalt. The seemingly simple measurement of one barite sample can be argued to give a better estimate of the $^{87}\text{Sr}/^{86}\text{Sr}$ of the Earth's mantle 3.45 billion years ago than was previously available from direct measurements on contemporaneous volcanic rocks.

McCulloch then argues that his measurement puts limits on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio that the Earth could have had when it finished accreting from solar nebular material. Specifically, he argues that the Earth must have continued accreting until 4.48 billion years ago in order to achieve such a high ratio of $^{87}\text{Sr}/^{86}\text{Sr}$, and at that time the Earth's $^{87}\text{Sr}/^{86}\text{Sr}$ ratio was much higher than the Moon's. Actually, his data can be reinterpreted as indicating that the mean time of Earth accretion was 4.48 billion years ago, and therefore that the end of Earth's accretion must have been even later. Although McCulloch's conclusion requires some assumptions, he has made his assumptions conservative enough that the conclusion appears robust.

The strontium ratio of the early Earth and the rubidium/strontium ratio of the Earth are particularly significant for the origin-of-the-Moon issue. The Rb/Sr ratio

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

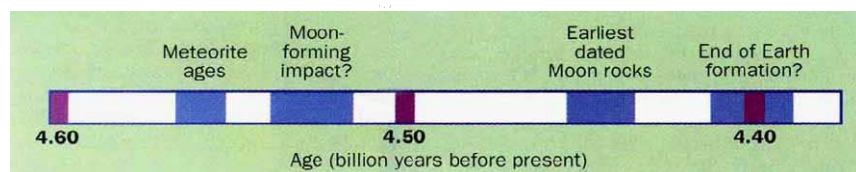
Earthrise from the Moon.

reconcile with the arguments put forward by McCulloch, unless the Moon is made up mainly of material from the impact body rather than from the Earth itself. This idea has its own problems, however, because it implies that the otherwise similar aspects of lunar and terrestrial geochemistry must be coincidental⁵.

It is tempting, of course, to argue that the constraints described by McCulloch are not very strong and should be discounted. However, his result is consistent with other evidence that the Earth had a high initial value of $^{87}\text{Sr}/^{86}\text{Sr}$. In virtually all old terrestrial rocks, ranging in age from 1.8 to 3.5 billion years, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are too high to be compatible with the idea that the Earth has a low, Moon-like initial value of $^{87}\text{Sr}/^{86}\text{Sr}$. Conservatives might argue that the high $^{87}\text{Sr}/^{86}\text{Sr}$ values found in old terrestrial rocks are not reliable, that they have been modified by subsequent processes. The problem with ignoring the existing data, however, is that a very large number of rocks have been studied over the past few decades, and no trace of the expected low $^{87}\text{Sr}/^{86}\text{Sr}$ values has been found.

The new barite measurement highlights the large degree of interrelation between models of Earth evolution, lunar origin and early Solar System evolution. Someone needs to find the elusive low- $^{87}\text{Sr}/^{86}\text{Sr}$ values in the Earth, or we are left to face the implications as described by McCulloch — a younger Earth, and an early giant impact. □

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As usual, however, the devil is at work in the details. If the Moon is made of Earth material, then one would expect the Moon to be similar in chemical composition to the outer parts of the Earth. Although it is indeed broadly similar⁴, it differs in important ways, and proponents of the impact theory need to add some significant twists to the story to make it compatible with the geochemical differences between the Earth and Moon.

Malcolm McCulloch's new research¹ adds further fuel to the debate. McCulloch measured the strontium isotope composition of a barite (barium sulphate) deposit in Australia that is 3.45 billion years old. The barite measurement is significant because barite is a marine precipitate, and therefore its $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can be argued to be representative of the $^{87}\text{Sr}/^{86}\text{Sr}$ of the world's oceans 3.45 billion years ago. The oceans, in turn, are likely to be representative of the Earth's mantle at that time because the oceanic strontium budget would have been dominated by hydrothermal exchange with

is a representation of the primary chemical difference between the Earth and the Moon. Relative to the Earth, the Moon is greatly depleted in 'volatile' elements (such as rubidium) in comparison with 'refractory' elements such as strontium. To make a consistent story from the impact theory, the Earth needs to have a low $^{87}\text{Sr}/^{86}\text{Sr}$ and Rb/Sr until the Moon forms, and then acquire additional Rb and ^{87}Sr -rich material so that it can end up having a higher Rb/Sr and a higher $^{87}\text{Sr}/^{86}\text{Sr}$ than the Moon. Although the picture becomes complicated, McCulloch's result implies that the Moon-forming giant impact probably occurred very early while the Earth was much smaller than it is now. Subsequently, the Earth must have continued growing to its present size, somehow accumulating new material that was able to bypass the nearby Moon.

Proponents of the giant impact theory generally believe that the impact occurred late in the accretion history of the Earth, when the planet was very close to its present size. A late impact is difficult to

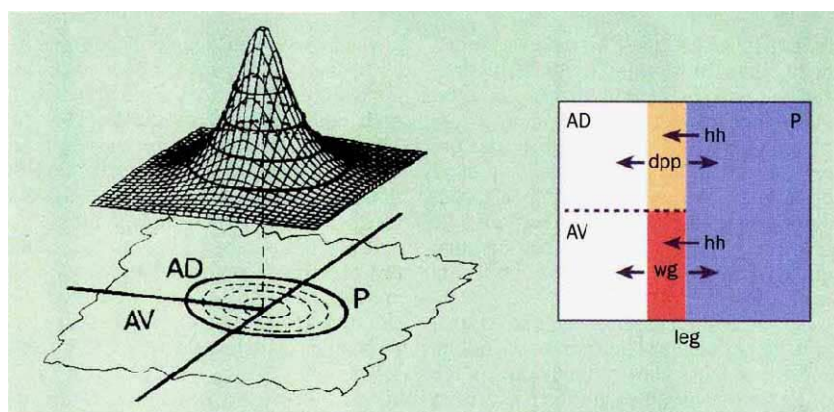
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It takes three to distalize

Jean-Paul Vincent and Peter A. Lawrence

THREE secreted proteins of *Drosophila* are now in the spotlight. These stars, the products of the *hedgehog*, *wingless* and *decapentaplegic* (*dpp*) genes, act individually or in pairs at various places in the developing fly. Here we pick out one situation in which they appear together on the same stage. As shown in two recent papers^{1,2} and by Diaz-Benjumea *et al.*³ on page 175 of this issue, the three molecules cooperate to determine the position of the prospective distal tip of the *Drosophila* leg. From here the proximo-distal axis is

It is precisely from this meeting point that distal structures (such as the terminal claws) arise and from here that the proximo-distal axis is organized. Campbell *et al.*² first proposed that the meeting between *wingless*-expressing and *dpp*-expressing cells would be sufficient to produce a proximo-distal axis. To show this, they used the *flp*-out technique invented by Struhl and Basler⁸ to make discs express *wingless* uniformly. In such discs, *Aristaless* protein (a marker of the leg tip), instead of being confined to the



Juxtaposition of three compartments (posterior (P), antero-dorsal (AD), antero-ventral (AV)) generates a distalizing centre in an insect leg. Hedgehog produced in the posterior compartment (blue) activates expression of *dpp* (yellow) and *wingless* (red) in the antero-dorsal and antero-ventral compartments. Meeting of *wingless*- and *dpp*-expressing cells leads to a distal tip. (Left, from Meinhardt⁹; right, adapted from Basler and Struhl¹.)

set up and the leg grows out.

Drosophila leg precursors (imaginal discs) can be subdivided into three domains according to the expression pattern of — or the competence to express — these secreted proteins¹. The three domains could well be coextensive with compartments of cell lineage that subdivide leg discs and, although this has not yet been proved, we will take it for granted for now. In this view, all posterior compartment cells express *hedgehog*⁴, all antero-ventral cells are competent to express *wingless*, and all antero-dorsal cells competent to express *dpp*¹.

The *engrailed* selector gene specifies the posterior compartment⁵ and, among other things, directs *hedgehog* expression in all the posterior cells^{6,7}. When Hedgehog protein spills over into the anterior compartments from the posterior, it activates *wingless* in the antero-ventral compartment and *dpp* in the antero-dorsal¹. Because the Hedgehog protein does not spread far, the zones of expression of *wingless* and *dpp* are confined to narrow bands of cells that run along the antero-posterior compartment border and meet at the centre of the disc (see figure).

centre of the disc, is now found in a long stripe where *dpp* is normally expressed, along the dorsal portion of the antero-posterior compartment border. This gross expansion of the tip domain leads to abnormal appendages while, as shown originally by Struhl and Basler⁸, smaller and isolated clones of ectopic *wingless*-expressing cells in the antero-dorsal compartment lead to axis duplications.

Diaz-Benjumea *et al.*³ now provide further evidence that *wingless* and *dpp* are necessary and sufficient to induce distalization. First, they remind us that in the absence of either protein, no distalization occurs. Second, using the same *flp*-out technique, they force *dpp* expression throughout the disc. The result is, again, an expansion of distal fates in a territory that coincides with the normal domain of *wingless* expression (this time another marker of distal structures, the *Distal-less* protein, is used). Small clones of *dpp*-expressing cells induce duplicated axes (albeit incompletely distalized) when they overlap with the normal *wingless* expression domain.

From the three papers, then, it seems that distalization occurs in two steps.

First, Hedgehog induces expression of *wingless* and *dpp* in domains that meet at one point¹. Second, the combined action of *Wingless* and *Dpp* around the meeting point somehow results in the formation of a distal tip. Cells of the prospective distal tip then begin to express genes such as *aristaless* and *Distal-less*^{2,3}.

In compartment lingo, we would say that interactions between cells of different genetic addresses establish the proximo-distal axis: first across the antero-posterior compartment boundary and then across the border between the antero-dorsal and antero-ventral compartments. It is remarkable that this scenario was anticipated by Meinhardt more than ten years ago⁹. His model was based on the simple realization that a point is uniquely specified by two intersecting lines, and he proposed that the compartment boundaries were two such lines which crossed to specify the distal tip. His diagram of distalization is fundamentally similar to the drawing of Basler and Struhl¹ depicting the expression domains of *wingless* and *dpp* (see figure). In effect, Meinhardt's geometric outline has now been filled in with details of a mechanism by which cells of different compartments cooperate to secrete molecules that act at short range across compartment borders, reaching cells with different genetic programs and responses¹.

Over the years, Meinhardt's view of distalization has received less attention than the polar coordinate model. According to this model, imaginal discs are organized radially with a range of circular positional values (12 values are usually drawn to give the familiar clockface representation). One rule of this model is that discs form distal structures only when the full complement of circular values is present¹⁰. If sectors of discs with particular circular values are removed surgically, they must be regenerated before distalization can occur. It seemed to Meinhardt that three 'values' would be sufficient (the posterior, antero-ventral and antero-dorsal compartments). He argued that this simple compartment model of distalization could perform all the feats normally credited to the more baroque polar coordinate model and explain, for example, the complex axial symmetries of supernumerary legs. The three papers we

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have been discussing give a mechanistic edge to Meinhardt's view and make the polar coordinate model look like an unnecessary abstraction.

So, are the three genetic stars for real? Will they be remembered 20 years from now as the great actors who shot the clock at High Noon? We think they will: the misexpression studies using the *flp*-out system demonstrate that *wingless* and *dpp* do not just correlate with axial patterning, they show that the genes are instrumental.

ELECTRONICS

Molecular wires and girders

Devens Gust

CIRCUIT boards in an electronic device have the obvious function of establishing electrical connections between components. But they serve another important role: preventing short circuits that would make the apparatus useless. Molecular electronics will likewise require not only molecular-scale switches, transistors and logic gates, but also nanoscale interconnections that provide essential electronic communication between components while blocking undesirable interactions. There are many approaches to the search for molecular 'wires', and two new reports^{1,2} deal with one of these: photoinduced electron transfer down a rigid link between a donor and an acceptor complex.

At the molecular level, transfer of an electron requires overlap of donor electronic orbitals with those of the acceptor. As orbitals decay roughly exponentially with distance, this overlap is a strong function of separation. But the overlap can effectively be extended if donor and acceptor orbitals overlap with those of intervening material, much as electrical connections between macroscale components are established by metallic wires. So molecular electricians are seeking nanoconductors that confer structural rigidity, preventing undesirable spatial arrangements that lead to molecular short circuits. Lehn *et al.* have linked two metal complexes with a conjugated polyene spacer and observed long-range electron delocalization¹, whereas Barigelli *et al.* have detected electronic interactions between complexes joined by rod-like polyphenylene groups².

The highly coloured carotenoid polyenes have long been known to be key players in photosynthesis. Photosynthetic reaction centres are molecular-scale photovoltaics that carry out charge separation across lipid bilayer membranes. So it was reasonable for Platt, following a comment by Strehler, to sug-

Indeed, antero-dorsal leg cells are fooled to act ventral when forced to express *wingless*. Likewise *dpp*-expressing cells become antero-dorsal irrespective of their positions in the anterior compartment. *hedgehog* definitely deserves the Oscar for best supporting actor as without it the two other stars would never meet. □

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gest in 1959 that carotenes might mediate electron transport across the insulating membrane³. This turned out to be wrong, but the basis of a good idea. Polyacetylene, an indefinitely extended carotenoid polyene, has been investigated as an organic conductor, and carotenoids have been used as electron transfer agents in solution and in synthetic membranes.

In the work of Lehn and colleagues¹, the polyene spacer in molecule **1** interconnects two optically and electrochemically

coupling in this case is estimated to be $2,100\text{ cm}^{-1}$ (0.26 eV).

The polyene linker in **1** acts not only as a molecular wire, but also as a spacer. It prevents folding motions that would allow the ruthenium complexes to come into van der Waals contact. The resulting strong electronic interaction would be the molecular equivalent of a short circuit.

This mechanical role of the polyene is aided by its conjugated backbone. The formally single bonds in the chain have some double-bond character due to overlap of adjacent π -electron systems. Rotation about these bonds involves orthogonal orientations in which the stabilizing overlap is lost, and rotation is therefore restricted. In addition, planar *s-cis* arrangements about the single bonds are sterically unfavourable. The result is a rigid molecular girder, at least on the timescale of rapid electron transfer reactions, which often take less than a nanosecond. (Nonconducting molecular girders also exist⁴.)

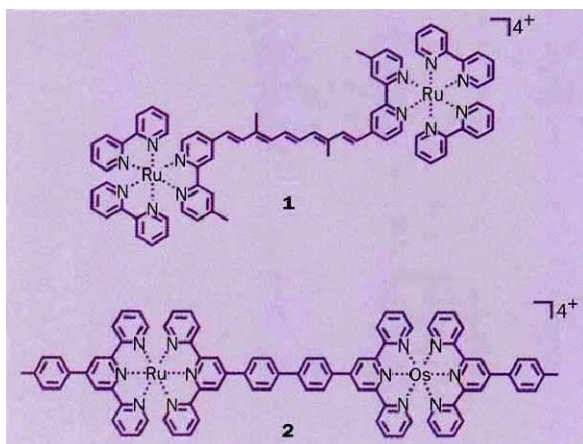
Carotenoids may also act as skeletal members in photosynthetic antennas. One such protein, the chlorophyll *a/b*-protein light-harvesting complex contains two molecules of the carotenoid lutein. The ends of each carotenoid make strong hydrogen bonds with polypeptide loops

on opposite sides of the photosynthetic membrane spanned by the complex. The polyene chains form an X-shaped cross-brace that presumably provides structural stability⁵.

At first sight, the work of Barigelli *et al.* looks similar. The ruthenium(II) and osmium(II) complexes of **2** are joined by a polyphenylene spacer². The structural similarity of polyphenylenes to polyenes suggests that they might also provide a conjugated pathway that could act as a molecular wire. However, steric interference between the hydrogen atoms on

adjacent phenyl rings prevents the rings from lying in the same plane, and thus limits conjugation. So Barigelli *et al.* used energy transfer experiments to probe the degree of electronic interaction through the polyphenylene chain in **2**. Although ruthenium complexes generally produce strong, long-lived luminescence, the ruthenium moiety of **2** showed no emission at all on excitation — instead, luminescence was seen from the osmium complex. This is proof of the transfer of excitation energy from the ruthenium complex to the osmium complex. The energy transfer rate constant is at least $5 \times 10^{10}\text{ s}^{-1}$.

In general, electronic energy transfer can occur by either the Förster dipole-dipole mechanism or the Dexter electron-exchange mechanism. The Förster pro-



active moieties. Although separated by 24 Å, the two ruthenium complexes do not act independently. The electronic absorption spectrum of the mixed valence (Ru(III)–Ru(II)) complex prepared from **1** shows an intervalence absorption band arising from optically promoted transfer of charge between the metal centres. Spectroscopic data allow calculation of an electronic coupling equal to 140 cm^{-1} (0.018 eV), which is consistent with the polyene acting as a conductive wire joining the two complexes. The effect is larger in the first excited triplet state of **1**. The triplet spectral properties suggest a state wherein an electron has been promoted into an extended π -molecular orbital embracing the polyene itself; the electron is delocalized between the linked bipyridyl ligands. The strong electronic

cess may be thought of as a coulombic, through-space interaction in which the excited chromophore acts as a transmitting radio antenna, and the acceptor chromophore as a receiver. The Dexter mechanism, on the other hand, requires overlap of the molecular orbitals of each pigment. It is basically a simultaneous two-electron exchange, and thus has much in common with electron transfer. Both types of energy transfer have been observed in photosynthesis and many other natural and synthetic systems.

In spite of the 20 Å distance between the metal centres in **2**, the energy transfer rate constant is one thousand times larger than that for related chromophores separated over 17 Å by a rigid but nonconjugated bridge. The rate is substantially faster than predicted by the Förster theory, so it seems the energy transfer must follow the Dexter mechanism, with electronic coupling through the polyphenylene spacer. Perturbations in the absorption spectra and electrochemical properties of the complexes back this up, again indicating that there is electronic interaction between the widely separated metal centres.

Molecular 'girders' of the polyphenylene type can strictly control the spacing (but not the angle) between groups linked to either end. The use of pyridine moieties at each end of the polyphenylene bridge allows ready attachment to metal centres. In principle, these terminal ligands could be 2,2'-bipyridine, as in **1**. But the use of substituted bipyridine ligands in complex optoelectronic molecular devices can lead to a plethora of unwanted stereoisomers, due in part to the chiral nature of the *tris*-bipyridyl complex. This problem does not arise with the tridentate terpyridine-type ligands in **2**, which form achiral complexes. Such ligands are likely to prove valuable for the preparation of many other molecular-scale devices.

Although convenient and functional molecular wires and girders are not yet routinely available, their development continues apace, and the recent results are encouraging. As the allusions to photosynthesis illustrate, biological systems have already discovered many of the principles now being exploited for molecular electronic applications. They could do much to guide the evolution of the field. □

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Symmetry without fear

Mark Kirkpatrick and Gil G. Rosenthal

WHY does nature love symmetry? The answer proposed by Enquist and Arak¹ on page 169 and Johnstone² on page 172 of this issue implicates a conspiracy between geometry and neural architecture that makes symmetrical visual patterns preferable to asymmetrical ones. Their idea provides a new explanation for why females of some animals favour mating

has the ability to recognize.

The new papers use this model to make an interesting point. Once trained, networks respond most strongly to the more symmetrical variants among the target set of images. Johnstone selected a neural network to distinguish images of an abstracted bird tail from random images. The images were not uniform: the tails varied with respect to their symmetry. After the network evolved to recognize them, Johnstone found that the most symmetrical tail elicited the network's strongest response even though the selection process gave no advantage to that image over the asymmetrical variants.

Enquist and Arak added another dimension by allowing for the evolution of the signal itself. They simulated a selection-mutation cycle in the image concurrent with that in the network. These workers also varied the projection of the image on the retina, allowing for both translation and rotation in the plane. Their most striking finding is that co-evolution of the signal and receiver results in signals with and preferences for rotationally symmetrical patterns.

Both studies are motivated by results showing that females have mating preferences for symmetrical males^{4,5}. The interpretation attached to those findings was that preference for symmetry evolves to identify males of high genetic quality. Symmetry is an indicator of good development and hence good genes, the argument goes, and females benefit evolutionarily by mating with these males and passing the good genes on.

The twin pillars of this argument are venerable but not yet proven. Minor variation in symmetry (known as a 'fluctuating asymmetry') has been discussed for over 30 years (reviewed in ref. 6), but its genetic, developmental and evolutionary significance remains obscure. Its relevance to sexual selection is a recent and controversial suggestion. The view that

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Symmetrical show — a male Temminck's tragopan on display.

with symmetrical males rather than their lopsided competitors. Although the results may not explain the fearful symmetry of William Blake's tyger, they have sundry implications for the evolution of animal communication.

The kernel of the new result is that artificial neural networks selected to recognize images tend to develop biases or preferences for symmetrical patterns. In these digital simulations of a visual system, the ability to recognize is acquired by a process that explicitly mimics biological evolution and that is also a model for learning³. Several networks that vary slightly are challenged with a set of target images to be recognized (say a bird's tail) and alternatives to be rejected (random patterns). The network that is most successful is retained and the others deleted. This step is analogous to natural selection: individuals that don't identify and mate with their own species leave no offspring. The surviving network is then used as a template for the next generation of networks. Random variations are added to these progeny, however, in analogy with genetic mutation. The cycle of selection and mutation is repeated many times, and a network ultimately evolves that

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female mating preferences have evolved to identify good genes entertains several alternatives. The new neural-network results show that preferences for symmetry can also arise as a simple by-product of how image recognition works. This view is consonant with theoretical results showing that mating preferences can be easily established as side effects of other evolutionary processes⁷. Enquist, Arak and Johnstone have thus provided a plausible alternative to the good genes interpretation of preferences for symmetry.

The underlying mechanism for the results obtained from neural networks involves a form of spatial averaging. Symmetrical images enjoy greater similarity with the other members of the target sets used in these simulations than do the asymmetrical images. Thus the preference for the average image comes from interpolation to the centre of the range of acceptable variants. This is the converse of an effect discovered earlier by Enquist and Arak⁸. They showed that a neural network which they selected to identify a single image responded even more strongly to some images that the network had never before seen. When it was selected to identify a tail of a certain length, tails that were much longer elicited a greater response. This behaviour results from extrapolation: like meteorologists and economists, neural networks respond unpredictably to stimuli outside their range of experience. The old and the new work do, however, convey the same moral: mating preferences can be established as a side effect of neural wiring.

The new results of course raise questions. One is whether preferences for symmetry emerge in visual systems, artificial or not, involved in recognizing three-dimensional objects. The current simulations confine images to rotation, translation and variation in a plane. But visual signals are viewed from various perspectives in three dimensions. It is not clear whether projections of three-dimensional images onto two-dimensional retinas might also produce inherent preferences for symmetry (see ref. 9). A second issue is whether preferences that neural networks may have for average images can be used to explain nuances of human visual aesthetics, as Enquist and Arak suggest.

Experiments with real animals would shed light on the question of whether the computer simulations reflect the behaviour of living sensory systems. Behavioural experiments could establish whether learning processes lead to recognition biases that favour symmetrical shapes. Artificial selection on innate visual-recognition mechanisms could determine whether genetic evolution does or does not follow rules similar to learning.

Mating displays are not the only type of signal that should converge on symmetry under the Enquist-Arak/Johnstone

hypothesis. Warning colour, seen for example in many species of unpalatable insects, should also evolve towards symmetrical patterns. Since a bilaterally symmetrical pattern is often the default for developmental reasons, a stronger test would be to ask if these aposematic patterns show stronger rotational symmetries than colour patterns not involved in signalling. And then there is camouflage, a non-signal. If these workers have it right, then Blake got it wrong. The tiger, seeking refuge from detection, is fearful for all its asymmetry. □

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SURFACE CHEMISTRY

Surmounting the barriers

Peter J. Feibelman and John Harris

DEVELOPING a materials science of 'heterogeneous catalysis' (catalysis by powdered solids) has been a long-term dream of the surface-science community. The motivation is plain. Catalytic chemistry already generates many billions of dollars a year in the world economy. A detailed, atomic-level understanding of surface reactions might permit the design of

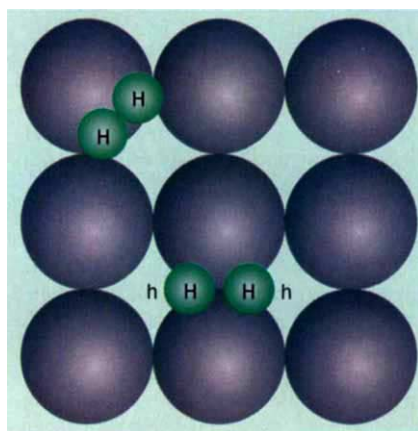
to molecular orientation and point of impact on the substrate lattice.

Surface chemical reactions relevant to industry can be complex, but some are rather simple, such as $\text{CH}_4 + \text{H}_2\text{O} \rightarrow 3\text{H}_2 + \text{CO}$, the 'steam reforming' reaction, which is catalysed by several transition metals, notably nickel and platinum. The rate-limiting step in steam reforming is believed to be the initial breakup of the very stable and compact methane molecule into CH_3 and H radicals that can readily participate in further reactions. Accordingly, a vital function of the catalyst in such cases is to facilitate molecular fragmentation.

It is no accident that catalysts are required to break up strongly bound species such as CH_4 . Such molecules are abundant in nature precisely because they are stable against naturally occurring processes. It is well known, however, that transition-metal surfaces and clusters composed, for example, of Pt, Ni or Fe atoms (which do not ordinarily occur in nature) are effective in dissociating relatively stable molecules. The reason that they are effective became clear as a result of asking why molecules that dissociate spontaneously on these metals do not do so on all metals⁴.

There are two key ideas. The first is that an H_2 molecule can dissociate only if it lies close enough to metal atoms (M) that formation of M-H bonds is energetically favourable (that is, close enough that two M-H bonds can form that, taken together, are stronger than the H-H bond). The second idea is embodied in the shell structure of atoms and molecules: that closed or complete electronic shells are electronically rigid.

Spontaneous dissociation requires the H_2 to approach a metal closely without expending appreciable energy to do so. When a closed-shell molecule such as H_2 or CH_4 approaches a metal surface, it first encounters the surface's most loosely bound electrons. This occurs far enough from the metal atom cores that strong M-H bonds cannot yet form. So unless the loosely bound metal electrons can 'get out of the way', the molecule is repelled. This



Top view of a face-centred-cubic metal crystal surface. The large circles represent the metal atoms. The smaller circles marked 'H' are hydrogen atoms bound in a molecule. The sites marked 'h' are the hollow sites which single isolated H-atoms prefer on the surface. In the bottom half of the figure the molecular orientation is such that the H-atoms are 'aimed' at h-sites. In the upper half, the molecule is shown in its preferred orientation immediately before dissociation. The H-atoms do not point towards the optimal h-sites, but to bridge sites. The reason is that this orientation allows metal-hydrogen bonds to compete better with the H-H bond in H_2 (see text).

less expensive, more effective catalytic substrates.

The past decade has seen great strides towards such an understanding, largely through a deliberate focus on the dissociation of H_2 , the simplest example of molecular fragmentation on a surface. Early work concentrated on the basic features of the process, but a series of reports in *Physical Review Letters*¹⁻³ testifies to increased sophistication, exploring fine details of the reaction path such as sensitivity

is a consequence of Pauli repulsion — the fact that a closed electron shell repels any other electrons. At transition-metal surfaces, the most loosely bound electrons can get out of the way of the incoming H_2 by converting into d -electrons that are strongly localized about the metal atom cores. This is impossible on simple metals (Al, Mg) which have no d -levels, and on noble metals (Cu, Ag, Au) whose d -shells are full. But it is possible on transition metals, because their d -electron shells are 'open', or incomplete.

This argument qualitatively explains the catalytic inactivity of the simple and noble metals relative to the transition metals. But what about the optimal reaction path and the variation of forces as the molecule is rotated or translated away from this path, both significant in calculating the reaction rate under thermal conditions? The new developments¹⁻³ bear on these important details.

Cohen and colleagues¹ begin from the earlier discovery that the optimum path does not necessarily correspond to a molecular orientation and impact site such that the dissociation fragments are aimed at the locations where they will optimally reside on the surface. Specifically, calculations for a rhodium surface⁵ revealed that the favoured reaction path is asymmetric with respect to the surface lattice, with the H-atoms aimed at sites where they bridge two Rh-atoms. Only after the H_2 dissociates will the H-atoms move to the most favourable locations for chemisorbed atoms, the hollows marked 'h' in the figure. The 'obvious' dissociation route, shown in the lower part of the figure, is less favourable because it requires additional stretching of the relatively short H-H bond, at additional energy cost, before strong M-H bonds can form.

Whereas this conclusion was based on explicit energy and force calculations, Cohen *et al.* seek to develop an approximate theory based on the notion of a surface response function, a 'softness' or local electronic polarizability, which directly expresses the ease of promoting the looser metal electrons into strongly localized d -levels. To illustrate their approach, Cohen *et al.* show that a count of unfilled d -levels correlates with the optimal H_2 dissociation geometry found in ref. 5 for Rh(001). Although the predictive power of this softness function approach remains to be demonstrated, it could be a step on the path towards a 'frontier orbital' picture for surfaces^{6,7} that quantitatively correlates catalytic activity with clean surface attributes.

Direct calculations of molecular dissociation trajectories at surfaces have awaited accurate, fast computational methods for calculating forces. Accuracy is an issue because until recently no method was available that allowed absolute dissociation energies to be evaluated

adequately for systems of more than a few atoms. Speed is a concern because even H_2 -dissociation involves at least six degrees of freedom (three coordinates per H-atom, more if a thermal ensemble of metal lattice configurations is considered). The computational effort required obviously scales up with the dimension of the configuration space that must be explored.

Recent progress alleviates both problems. Density-functional calculations⁸, which have for years been used to predict accurate structures of molecules and solids, now incorporate corrections that allow evaluation of dissociation energies to a few kilocalories per mole (in many cases). At the same time, computing costs have dropped astonishingly. Inexpensive work stations and massively parallel computers make yesteryear's 'supercomputing' commonplace. This progress, together with methodological and algorithmic improvements (for example ref. 9), brings within the reach of first-principles calculations problems that only a decade ago were regarded as hopelessly inaccessible.

Simultaneous articles by Hammer *et al.*² and White *et al.*³ use modern hard- and software to arrive at the first detailed, global potential-energy surfaces for H_2 dissociation on a metal (Cu). Both calculations agree with the experimental conclusion that H_2 dissociation on Cu requires overcoming an energy barrier in the range of 0.5 to 1 eV. They also agree that this barrier is extremely corrugated along the Cu surface, and strongly dependent on molecular orientation.

Dynamical simulations of H_2 -Cu scattering processes remain to be carried out using the new potential-energy surfaces. When their results are compared to the many available experimental data on this system, we will know just how close we have come to having the important details of H_2 -dissociation in our grasp. In any event, the present series of papers promises a new era in the theory of surface chemical reactions. □

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Forever airborne

THE wandering albatross spends its time at sea, gliding over the crests and in the troughs of the waves. It seldom has to put power into its wings. Instead, it exploits the updraughts from wind streaming over the wave crests. It is, in fact, a flying machine powered by the interactions between waves and wind. In a flat calm, it has to rest on the water until active weather returns.

Daedalus wants to imitate this trick. He is designing an artificial albatross. It is essentially a fast-acting, variable-geometry glider. Tiny anemometers monitor the air currents flowing around it, and a small Doppler laser system scans the surrounding waves and their movements. An on-board computer processes these data and operates the control surfaces, so as to exploit those updraughts that permit the best progress in the programmed direction. Its systems will be powered by a small windmill rotor driven by its slipstream. It will be quite self-contained; over an active sea it will stay airborne indefinitely.

Much development work, both in hardware and software, will be needed to produce a working artificial albatross. It will never fly as gracefully as the real thing, but will share its limitations. In a flat calm, it too will have to come down onto the water. The subsequent take-off may be difficult. Daedalus plans to give the craft some sort of compressed-air jet device to punch it vertically aloft when the sea is active again. Once airborne, it will slowly recharge the jet reservoir with power bled from its windmill rotor, ready for the next ditching. In very rough weather, even real albatrosses sometimes get hit by waves, and have to recover from the blow and take off again from the churning water. This would be fatal for the artificial albatross. In a storm it will have to abandon its programmed course and simply keep as high as possible above the turbulent surface.

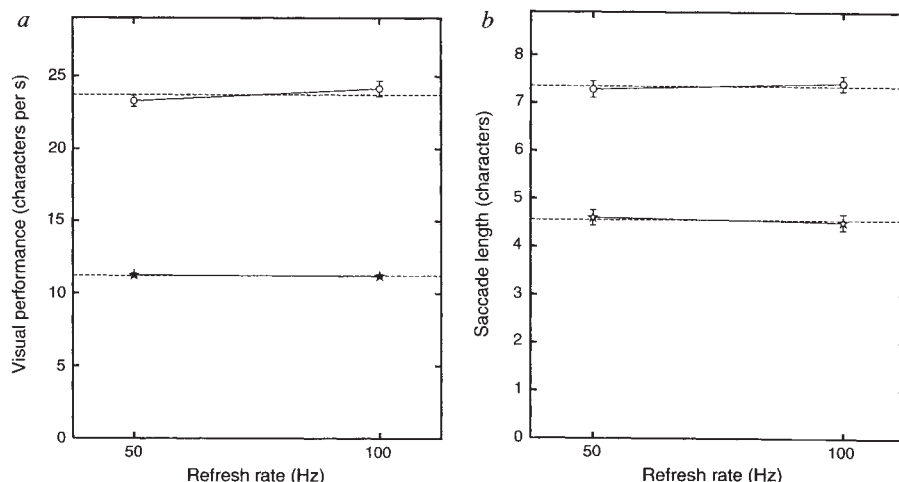
But with all these limitations, it will still be a wonderful oceanographic tool. Artificial albatrosses will quarter the oceans on tours of duty lasting months and spanning the globe. They will make close measurements of wave patterns, surface temperatures, oil slicks and so on, while satellites overhead track them and receive their findings, and relay fresh orders. Meteorologists will welcome their detailed accounts of remote wind and weather, navies will deploy them in antisubmarine warfare, and biologists will use them to shadow turtles and eels on their mysterious migrations. They might even be used to shadow real albatrosses, provoking interesting confrontations of rivalry or desire.

David Jones

Reading and screen flicker

SIR — We were motivated by the Scientific Correspondence¹ by Kennedy and Murray, who suggested an adverse effect of higher CRT-display refresh rates on saccadic extent and performance during reading, to investigate the problem further. Kennedy and Murray's experiment deviated extensively from normal reading, as only three separate words were involved in a two-alternative

facilitate correction for learning effects during the search experiments. In the other task, reading velocity is registered while subjects read simple Dutch texts containing 9 lines each. Two conditions are considered: applying a 100-Hz digital scan (Philips 28PT910A) and a 50-Hz (Philips 28PV7805) television set. Both sets are calibrated⁴ to a comfortable luminance contrast ratio of 4.0, applying an



a, Visual performance versus image refresh rate. Reading and search velocity expressed in number of scanned characters per second. Height of error bars (reading) and star symbols (searching) indicates twice the standard error. The large difference between the two measures is explained by the difference in redundancy and work load that exists between the tasks. The search velocity results are the average of 20 subjects performing 18 counts per condition (stars); the reading results are the average of 4 subjects who read 25 texts per condition (open circles). *b*, Length of progressive saccades expressed in number of spanned characters versus image refresh rate. The difference between the results of the tasks is as explained above. The results are the average of 4 subjects counting 12 pseudo-texts (stars) and reading 25 texts (open circles) per condition.

forced-choice task. It is known that each word has an optimal fixation position² from which only small deviations have a disproportionate effect on the recognition process³. Therefore, the conclusion of an adverse effect on reading performance would be justified if saccade length is really affected during the reading of a display with a higher refresh rate. Our aim was to investigate whether the length of progressive saccades and visual performance are influenced by image refresh rate.

We used two types of tasks in three experiments involving letter-search and normal reading. Search velocity registered in a letter-search task is known to be a sensitive performance measure that discriminates well between display conditions with respect to legibility factors^{4,5}. In this task, subjects scan pseudo-texts and search for target character 'A'. They are asked to press a button each time the target is spotted as a check on their accuracy. In the present case, pseudo-texts consisted of 9 lines with 30 character positions filled with random strings separated by spaces and containing 10–15 targets in total. A set of printed references is included to

extreme character luminance of 100 cd m⁻² and a background luminance of 25 cd m⁻². The character size is chosen to be comparable to television subtitles; for the letter 'X', this is 13.2 × 29.5 (width × height) min of arc at 240 cm viewing distance.

In the first experiment, search velocity is registered on 20 subjects performing a letter-search task for both conditions. This is repeated on four subjects in the second experiment simultaneously with eye-movement recording. Subjects' eye movements and reading velocity are registered during the reading task in the last experiment. After each session subjects are asked to point out the most comfortable display condition. The searching and reading velocities characterizing visual performance are shown in part *a* of the figure. Although a positive effect of higher refresh rates is suggested by the reading results, the effects on both measures are found to be statistically insignificant (reading: $F(1,5) = 0.58$, $P = 0.48$; searching: $F(1,18) = 0.32$, $P = 0.58$). In part *b* of the figure, the average extent of progressive saccades is plotted against

refresh rate. In neither case is the effect of refresh rate on saccade length statistically significant (reading: $F(1,5) = 0.29$, $P = 0.61$; searching: $F(1,3) = 0.58$, $P = 0.50$). In all cases subjects indicated the 100-Hz set to be the most comfortable display because of its 'flicker-free' image.

We conclude that visual disturbances that hamper the reading process, if present, are not different for the two refresh rates used. Further, regarding the subjective quality preferences, it is clear that the 100-Hz display is superior to the 50-Hz display.

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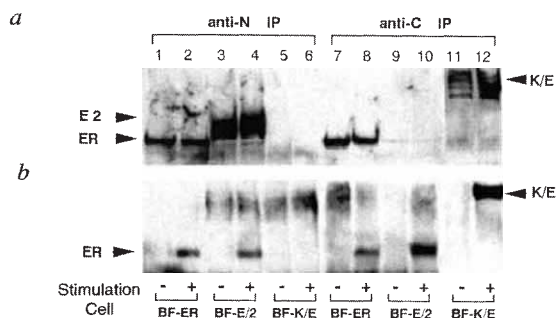
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Second subunit of Epo receptor?

SIR — Ligand-induced homo- or hetero-oligomerization of cytokine receptors triggers intracellular signal transduction pathways¹. Homodimerization of the erythropoietin (Epo) receptor cytoplasmic domain has been shown to be sufficient for growth and differentiation signals^{2,3}. However, Chiba *et al.*^{4,5} have published provocative data from which they concluded otherwise. In particular, their studies used chimaeric receptors containing the extracellular domain of the Epo receptor and the cytoplasmic domain of the interleukin (IL-2) receptor β -chain (E2) or the IL-3 receptor β -chain (E3). Because these receptors induced an Epo pattern of tryosine phosphorylation⁴ and erythroid differentiation⁵, Chiba *et al.* concluded that the Epo receptor extracellular domain interacted with other membrane components for signal transduction.

In my view, Chiba *et al.* did not address the phosphorylation of the chimaeric receptors or the possible expression of the endogenous Epo receptor in the cells. To explore these points, I used Ba/F3 cells expressing wild-type Epo receptor (BF-ER), the E2 chimaeric receptor (BF-E2) or a chimaeric receptor (kit/ER) with the extracellular domain of the oncogene *c-kit* and the cytoplasmic domain of the Epo receptor (BF-K/E).

I found that BF-ER and BF-E2 cells grew in Epo whereas BF-K/E cells grew in stem cell factor (SCF) as reported^{2,4}. As illustrated in the figure, an antiserum against the extracellular domain of the Epo receptor (anti-N) precipitated the M_r 66,000 (66K) Epo receptor (panel a, lanes 1,2) and the 70K–75K E2 (lanes 3, 4) but not kit/ER (lanes 5,6) whereas an antiserum against the cytoplasmic domain (anti-C) precipitated the Epo receptor (lanes 7, 8) and kit/ER (lanes 11, 12) but not E2 (lanes 9,10). With both antisera,



BF-ER and BF-E2 cells were stimulated with Epo and BF-K/E cells with SCF. Anti-N and anti-C immunoprecipitates (IP) from unstimulated (–) and stimulated (+) cells were immunoblotted with anti-N (a, lanes 1–10), anti-C (a, lanes 11 & 12) or anti-phosphotyrosine (PY) (b). Arrowheads, position of the wild-type Epo receptor (ER), the E2 chimaera (E2) and the kit/ER chimaera (K/E).

however, I detected a protein in BF-E2 cells that was the size of the wild-type receptor, consistent with the expression of the endogenous Epo receptor in these cells.

The expression of a wild-type Epo receptor suggests that the results of Chiba *et al.* can be explained by signalling through the wild-type receptor rather than the chimaeric E2 receptor. To explore this possibility, I next examined Epo-induced tyrosine phosphorylation of the chimaeric receptors as a marker for their use. Epo stimulation of BF-ER cells resulted in tyrosine phosphorylation of the Epo receptor (panel b, lanes 2, 8) as expected, while SCF stimulation of BF-K/E cells resulted in tyrosine phosphorylation of the kit/ER chimaera (lane 12). With BF-E2 cells, Epo stimulation did not induce detectable tyrosine phosphorylation of the E2 chimaera but did induce the tyrosine phosphorylation of the endogenous, wild-type receptor as detected by both anti-N and anti-C (lanes 4, 10). This indicates that the endogenous Epo receptor in BF-E2 cells is functionally expressed on the cell surface. This result also raised a concern about the functional integrity of the E2 chimaera. The level of tyrosine-phosphorylation of the wild-type receptor in BF-E2 cells was comparable to that in BF-ER cells, suggesting that the amount of cell-surface wild-type Epo

receptor in BF-E2 cells was similar to that in BF-ER cells. This might be due to an erythroid-like phenotype of BF-E2 cells⁵, because the endogenous Epo receptor in erythroid cells was efficiently expressed on the cell surface⁶, whereas ectopically over-expressed Epo receptor was mostly present inside the cells.

The results demonstrate that BF-E2 cells express and use the endogenous Epo receptor, and can explain the results by Chiba *et al.*, without hypothesizing another signal transducer. The basis for the high levels of expression of the endogenous receptor in BF-E2 cells is unknown. Because the parental cells barely express Epo receptor, it is likely that expression of the endogenous receptor is activated during selection. Alternatively, expression of the E2 chimaera may affect the expression or stability of the endogenous Epo receptor.

The above results may also bring into question the results by Chiba *et al.* with a chimaeric receptor (2E-R) containing the extracellular domain of the IL-2 receptor β -chain and the cytoplasmic domain of the Epo receptor. In particular, studies by Mori *et al.*⁷ had found that this chimaera, while binding IL-2, could not transmit a proliferative signal while Chiba *et al.* found that it could. It is possible that the endogenous IL-2 receptor β -chain was activated in the cells examined by Chiba *et al.* This is a particularly important point in view of the recent demonstration that the IL-2 and Epo receptors associate with and activate different Janus kinases⁸.

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TODOKORO REPLIES — Erythropoietin (Epo)-induced receptor phosphorylation cannot be detected by an immunoblotting of the total cell lysates with anti-phosphotyrosine antibody, because the level of receptor phosphorylation is too low, and thus the chimaera receptor phosphorylation was not shown in our paper⁴. Yoshimura claims that Ba/F3 cells expressing the E2 chimaera (BAF/E2) induce the expression of endogenous Epo receptor and thus that Epo stimulation induces phosphorylation of the Epo receptor, although it is not clear why endogenous receptor expression is induced.

In our hands, however, we cannot reproduce his results. As we have previously described, the E2 chimaera⁵ as well

as the Epo receptor^{5,9} in Ba/F3 cells transmit an erythroid-specific differentiation signal, that is, they induce the expression of the erythroid-specific transcriptional factors GATA-1 and SCL, which in turn induce expression of erythroid-specific genes such as globin and the Epo receptor. Therefore, once BaF/E2 cells as well as BaF/Epo receptor cells are stimulated with Epo, they are committed to erythroid-like cells expressing erythroid-specific proteins including the Epo receptor, and this process is irreversible. Yoshimura observed the endogenous Epo receptor because he used BaF/E2 cells cultured with Epo. But when we added Epo to transfectants that had not been cultured with Epo, we observed that both growth and differentiation signals were transmitted through the chimaera receptors but not through wild-type Epo receptor.

We also showed that the parental cells or the transfectants expressing inactive mutant Epo receptors would never spontaneously grow in response to Epo through the endogenous Epo receptor. Our previous papers simply suggested a possibility that there exists another subunit for Epo receptor^{4,5}. I agree with Yoshimura that homodimerization of the Epo receptor is necessary for signal transduction, but there is no evidence that it alone is sufficient. No one can explain why the crosslinking of the labelled Epo with Epo receptors always shows multiple bands, only one of which reacts to anti-Epo receptor antibody¹⁰; therefore, the possibility of a second subunit cannot be ruled out. There are several pieces of evidence supporting the existence of membrane proteins associated with Epo receptor^{10–17}, but in order to clarify this issue the second subunits must be isolated and characterized.

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Defenders of the faith

Michael Sharratt

Galileo for Copernicanism and for the Church. By Annibale Fantoli. *Vatican Observatory Publications*: 1994. Pp. 540. \$21.95 (pbk).

A Defense of Galileo, the Mathematician from Florence. By Thomas Campanella, OP of Calabria, translated with an introduction and notes by Richard J. Blackwell. *University of Notre Dame Press*: 1994. Pp. 157. \$27.95.

THE 'rehabilitation' of Galileo by Pope John Paul II in 1992 concluded the work of a group he had set up 11 years earlier to study the Galileo case impartially. The Pope's official and handsome apology for the notorious condemnation of 1633 did not, of course, exhaust the topic. So Annibale Fantoli's book on Galileo's work both for Copernicanism and for the Roman Catholic Church is not just tacked on to the *Studi Galileani* initiated by the papal commission, as though it had missed a deadline. That George V. Coyne, the Jesuit director of the Vatican Observatory, has translated Fantoli's original Italian volume, which appeared in the same series in 1993, may be a sign that the observatory will continue to encourage interest in Galileo.

This is not a full biography of Galileo or a complete survey of his contributions to science. Instead, it deals with Galileo's attempts to have Copernicanism at least tolerated and his consequent endeavours to save the Catholic Church from making a damaging mistake. So Galileo's work on mechanics is treated only at the general level necessary to understand his Copernican cosmology. Naturally, Fantoli is fuller on Galileo's work on astronomy, although even here he does not provide a specialist's contribution to the history of science; rather he highlights the ideological, conceptual and religious influences on Galileo. Within these limitations the book is a very useful account of the development of Galileo's thought.

That Jesuits figure prominently in the story is not due to fact that Fantoli himself was formerly a Jesuit but simply because they were the most influential Catholic teachers. To understand why Galileo, whether fairly or not, eventually blamed them for many of his troubles is an essential part of appreciating the context in which he worked.

The influence on the young Galileo of Jesuit teachers at the Roman College has come to light only in the past 25 years. Fantoli, along with many others, accepts William Wallace's thesis that Galileo had access to written notes of lectures given at the college. There is no mention of Alistair Crombie's argument that the undoubted Jesuit influence on Galileo

could have come from easily accessible printed works. If Crombie is right, then Wallace's dating of Galileo's early Aristotelian manuscripts would be considerably upset. Another omission in an otherwise well documented survey is the

IMAGE
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REASONS

Galileo before Pope Urban VIII, 1633.

work of C. B. Schmitt on the University of Pisa and contemporary Aristotelianism.

Fantoli is particularly good on Galileo's dealings with Jesuit astronomers, to whom Galileo turned to for help and cooperation after making his observational discoveries. We gain a proper understanding of why Galileo was initially wary of the mathematicians of the Roman College. Fantoli describes their public endorsement of the discoveries to which they were beginning to contribute; and he brings out well the limitations of Jesuit educational policy. One could say that for Galileo, novelty was attractive, almost a recommendation for an idea, whereas for the Jesuits novel opinions were suspect until proved harmless.

Fantoli traces the first signs of incipient alienation in the controversy between Galileo and Scheiner over sunspots. The disastrous intervention of Church authority is soberly set out, with welcome emphasis on Galileo's own attempt to provide a better way of interpreting scrip-

ture than that insisted on by Cardinal Bellarmine (another Jesuit). Equally even-handed is the treatment of the controversy with the Jesuit Grassi over comets, a controversy that led to Galileo's *Assayer* of 1623, itself a manifesto in favour of Galileo's way of doing science and against the Aristotelian way of the Jesuits and most universities. Finally the ill-fated *Dialogue* is usefully surveyed, although there could have been more emphasis on how much of it attempts to settle old scores with Scheiner.

Fantoli uses all this background (and a good deal more about other defenders of Aristotelianism) to take the reader through Galileo's trial and condemnation. He is judicious in his use of sources. In particular, he employs Grienberger's letters to telling effect to sift what is credible from the gossip of Galileo's friends. More than once he courteously dismantles a fragile conjecture of the late Stillman Drake. On those occasions when he blames Galileo for rash judgement, unfairness or arrogance he gives his reasons for doing so; but his admiration for a great scientist who was trying to save his Church from calamity comes out clearly, both in his chapter on the trial and in his comments on official Catholic attitudes to Galileo in modern times. The translation, apart from minor blemishes, is adequate. Despite Fantoli's modesty in describing his aims, this book is one that any specialist in the history of science could learn from.

In 1615, when Galileo was expanding an earlier essay on how to interpret scripture, he was encouraged by the publication of a similar essay by a Carmelite priest, Foscarini. Richard Blackwell included a translation in his *Galileo, Bellarmine, and the Bible* (University of Notre Dame Press, 1991). He has now translated the defence of Galileo written in 1616 by the brilliant although unfortunate Dominican priest, Tommaso Campanella. As this defence of Galileo's right to advocate Copernicanism was not published until 1622 and was in any case completed too late to prevent an official ruling that Copernicanism could be no more than a mere calculating device, it seems that Campanella's work was largely wasted. But it is still well worth having this short work made accessible. The only weakness is in the translator's introduction, where Aristotelianism is emphasized to such a great extent that Ptolemaic techniques are scarcely mentioned. The part of the introduction dealing with Campanella's defence of Galileo is succinct and helpful, and the translation and annotations are good. □

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Care for all

William Yule

Individual and Community Responses to Trauma and Disaster: The Structure of Human Chaos. Edited by R. J. Ursano, B. G. McCaughey and C. S. Fullerton. Cambridge University Press: 1994. Pp. 422. £60, \$89.95.

A New Species of Trouble: Explorations in Disaster, Trauma, and Community. By Kai Erikson. Norton: 1994. Pp. 263. \$28.99, £17.95.

As our television screens are once again filled with horrifying pictures of a stricken roll-on, roll-off ferry lying at the bottom of the sea entombing 900 souls, my thoughts go back to March 1987 when the *Herald of Free Enterprise* capsized outside Zeebrugge harbour. That disaster affected the lives of a great many survivors and changed my professional life dramatically. From then on, trauma and disaster became part of my everyday life. Before 1980, mental health professionals had not fully recognized the full extent of human reactions to acute traumatic events. Reactions are now known to include distressing intrusive thoughts about the trauma, avoidance of the associated emotions and physiological arousal to the point of not sleeping or concentrating. But once these predominant features were seen to form a syndrome, and the syndrome was given the fancy label of 'post-traumatic stress disorder' (PTSD), then it was suddenly recognized everywhere.

Not surprisingly, sceptics wondered where the syndrome had been during the world wars or when the *Titanic* sunk. Others began to mock the flood of 'disaster counsellors' who suddenly appeared as if from nowhere. So are we over-diagnosing conditions that, if left to themselves, would remit spontaneously? Are normal stress reactions being unduly medicalized?

These two volumes go a long way to exploring these issues and summarize important findings from the past decade

Trauma of disaster — the ferry *Herald of Free Enterprise*, which capsized outside the Belgian port of Zeebrugge in 1987, leaving 94 people dead and 405 survivors.

and more. The contributors to the multi-authored book come mainly from the armed forces. The military has been aware for decades that personnel under stress can react badly and has developed first-aid techniques for keeping them fit for action. In times of relative peace, many countries put their military mental-health specialists at the disposal of civilian authorities to help with natural and man-made disasters. This seems a cost-effective way of using scarce resources and one that ought to be used more widely. It is good that the authors can share the results of military research with the international scientific community.

There are many important messages in these well written contributions. Body-handlers need training to prevent distress. Training, or 'stress inoculation', reduces adverse reactions and would be of help to all emergency workers. Today's "Critical Incident Stress Debriefing", which involves talking people through the incident, clarifying what actually happened and educating them about normal psychological reactions to such events, was used during the Second World War and is effective as a secondary prevention method for emergency personnel. Effective social support for primary victims clearly provides most survivors with some protection against full-blown PTSD.

Chapters concentrate on a wide range of disasters from natural ones such as floods or earthquakes through manmade ones such as Chernobyl to the experiences of people on board a Gulf War hospital ship. The picture is inexorably built up that the more a trauma is viewed by the victim as being deliberately caused, and the greater the physical suffering and the threat of death, then the greater the level of distress. There is always an interplay

between the actual danger and the subjective interpretation of the threat that determines the level of stress.

Kai Erikson's volume nicely complements this academic, scientific review. He provides a much more personal feeling of how various communities can be struck by disasters of various types. We learn about the Ojibwa tribe of Canadian Indians who suffered when their fishing rivers were poisoned by mercury from an industrial spillage; citizens of Colorado who discovered that leaked petroleum vapours were invading their houses; and Haitian immigrants who had their savings stolen. Whole communities are seen to be devastated and the effects of disaster are shown to affect more than individuals.

So who needs a caring response? Few will fail to be moved by *A New Species* and many will be convinced by the arguments in *Individual and Community Responses*. People are often unable to cope with the overwhelming emotions that hit them unexpectedly when a disaster strikes. We have a good feel for which groups are most vulnerable; we are reasonably certain that early interventions that help survivors to express these emotions save later distress; and we are even beginning to develop a more effective range of interventions for those who do develop PTSD. As we watch the grieving communities in Sweden and Estonia, as we hear the harrowing tales of the few survivors, then we must conclude that we all need a caring response. It should come from the whole community, not only a few mental health professionals. □

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Autumn Books

Next week's issue contains *Nature's* Autumn Books supplement. Reviewers include Hans A. Bethe on David Holloway's *Stalin and the Bomb*, P. W. Anderson on Roger Penrose's *Shadows of the Mind*, Stuart Sutherland on Antonio Demasio's *Descartes' Error*, J. L. Cloudsley-Thompson on Edward O. Wilson's autobiography *Naturalist*, W. F. Bynum on Adrian Desmond's *Huxley*, Richard Davenport-Hines on Robert Rhodes James's *Henry Wellcome*, Adam Kuper on Pat Shipman's *The Evolution of Racism*, R. V. Short on Carl Djerassi's collection of essays *From the Lab into the World* and Harvey Brooks on Alan Bromley's memoirs *The President's Scientists*.

Science boldly popularized

Grace A. Wolf-Chase and
Leslie J. Sage

Gene Roddenberry: The Last Conversation — A Dialogue with the Creator of Star Trek. By Yvonne Fern, with a foreword by Arthur C. Clarke. University of California Press: 1994. Pp. 228. \$20.

GENE Roddenberry, the creator of the television series *Star Trek*, wanted his epitaph to be "he loved humanity", according to Yvonne Fern. His vision of the future of humanity continues to shine forth from millions of television screens daily, and it is the source of this vision that Fern explores through an extended series of conversations with Roddenberry, interspersed with quotations from his friends. Although Fern often alludes to *Star Trek* characters and episodes, her book is not another piece of fan literature. She asks probing questions, such as "What do you most fear?" or "What would you not want to lose?", to examine the thoughts, feelings and hopes of the man to whom *Star Trek* was real, and who had the rare ability to personalize his vision for millions of people. In the course of her conversations, we gain almost as many insights into the author's mind and character as we do into Roddenberry's. The reader may find this somewhat distracting, but Roddenberry himself requested that the book should be written as a dialogue. He remarks: "You can learn a lot about a person by the questions he asks of others. It shows what is in his own mind, what kind of quest he is on."

The people who will most enjoy Fern's book are those to whom *Star Trek* is more than mere entertainment; they will find her conversations with Roddenberry an exploration of themselves. On the other hand, it is difficult to see how anyone who is not a *Star Trek* fan could enjoy it. Those few who are simply unaware of *Star Trek* will find the allusions mysterious, although Fern attempts to address this by providing a basic introduction to *Star Trek* at the end of the book. People who are aware of *Star Trek* but are either indifferent to it or actively dislike it may find that they have little in common with the ideals portrayed, as Roddenberry put so much of himself into his creation.

Roddenberry repeatedly identifies himself in Fern's book with the character of Spock. Critical thinking and diversity were

central to his vision of the future. He tells us: "If there was one theme in all of *Star Trek*, it was that the glory of our universe is its infinite combinations of diversity". Wisdom is not only to appreciate another point of view, but to "take a positive delight when someone says 'I disagree with you because...'" This is the essence of science.

Scientific illiteracy is prevalent among the general public in the United States and other countries because, sadly, many students are taught what to think rather than how to think. The spirit of exploration, which is the inspiration of science, is too often absent. This illiteracy extends to our educators and therefore the next generation of students. If that tide has been stemmed somewhat by *Star Trek*, then Roddenberry deserves his epitaph. That *Star Trek* has become part of the vocabulary of almost every country in the

about the future, but his knowledge of physics was incomplete, and his assumptions were clearly wrong. Roddenberry's legacy is to ensure that even if a warp drive engine is impossible, there will still be people trying to build one. That is what it means to be human, and science is an intensely human activity. □

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Birdman

C. M. Perrins

Dean of the Birdwatchers. By William E. Davis. Smithsonian Institution Press: 1994. Pp. 234. \$29.95.

LUDLOW Griscom (1890–1959) was an American ornithologist whose name is known on both sides of the Atlantic. Interested in birds from a very early age, he came from a wealthy family who did not really approve of his going into ornithology and who seem to have cut him off financially. Although he held a plethora of 'jobs' with ornithological and conservation organizations, his professional life centred on just two institutions, the American Museum of Natural History, from 1916 to 1927, and then the Museum of Comparative Zoology at Harvard, where he was based for the rest of his working life. While at Harvard, he played a key part in setting up the Boston Museum of Science, a task that sapped a great deal of his energy for many years, even when his health was failing (a plaque there reads "Ludlow Griscom, who almost gave his life for the Museum"). Apart from work in the New England area, his principal contribution to science was his studies of the avifauna of Central America, a region he visited on several occasions.

During Griscom's early years, collecting birds was still much in vogue. Field identification of birds was considered to be wholly unreliable, so records were substantiated only by collecting specimens. Field guides were unheard of. Perhaps Griscom's greatest achievement was to be the prime-mover in changing that. By careful field observation he was able to show that one could identify birds in the field and that shooting them was unnecessary. His field trips spawned a great interest in birds, in particular contributing to an increased popularity in bird-watching that has lasted to this day. The first volume of Roger Tory Peterson's field guides appeared in 1934, in which its author pays fulsome tribute to Griscom for teaching him the concept of field marks. The part Griscom played in mak-

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Ground control — Roddenberry (rear) oversees the direction of *Star Trek: The Motion Picture* (1979).

world proves that Roddenberry succeeded where many educators have not.

The role of science fiction in popularizing science should not be underrated, as it often is. Roddenberry was one of the most successful popularizers of the twentieth century; as children of the 1960, our own fascination with science was partly shaped by *Star Trek*. Scientific elitism has no place in a community that depends on the public for financial support. Too often scientists fail to see science popularization as an important endeavour. They oppose science fiction because some aspect (for example, a warp drive engine) is physically impossible. In reality, they are eroding the base of their own support.

Sceptics should be reminded that "conclusive proof" that heavier-than-air flight was physically impossible was published in the late 1800s by Simon Newcomb. He was right, given the assumptions he made

ing fieldwork acceptable was so great that he acquired the nickname that gives the present book its title.

Griscom coupled his enormous enjoyment of birds with a strong sense of the need for conservation and played an important part in its development in the United States. The last years of his life were dogged by ill-health, mainly bad circulation, apparently as a result of heavy smoking — a habit he refused to give up. His condition led to a series of strokes, the first in 1950, but he battled on, continuing to work until almost the end.

This book is a useful, thoroughly researched account of the life and work of this dynamic and forceful man, with whom it was apparently not always easy to get along. It contains a valuable appendix to all his published works. □

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Limits to knowledge

Adam Schultz

Geophysical Inverse Theory. By Robert L. Parker. Princeton University Press: 1994. Pp. 386. £30, \$39.50.

It is comforting to some that the realm of quantum theory typified by Heisenberg's uncertainty principle can be placed in a box and neatly ignored by those concerned with phenomena on the scale of everyday experience. That modern inverse theory breaks down this artificial barrier, and extends the notion that there are intrinsic limits to knowledge on all scales, may prove disturbing, yet it stands as one of the most important achievements of mathematical physics.

Inverse theory provides a framework for solving the inverse problem: that is, determining the values of the parameters that describe a physical model given a set of measurements sensitive to these parameters. This generally also requires solving a forward problem — determining the values the measurements would have, given a model with a set of known values of the parameters. Solving the inverse problem is generally a much more difficult proposition than solving the forward problem, and the best one can usually hope for is to place bounds on the unknown parameters, rather than to solve for them uniquely.

An example of a forward problem is to determine the seismic waveforms on the surface of the Earth given a model of the Earth's elastic parameters and a known seismic source. The corresponding inverse

problem is to recover these elastic parameters given a set of observations of earthquakes on the Earth's surface.

The relationship between the unknown parameters and the observed data is often nonlinear and the data are typically inadequate and contaminated by noise. Inverse models are usually nonunique: an infinite number of different models may lead to exactly the same set of observations. This may be due to the underlying physics or to the inexactness and sparsity of observations.

This is a field where many different schools contend. Robert Parker's inverse theory is less statistical than, say, Albert Tarantola's, and *Geophysical Inverse Theory* is a view of the field from Parker's particular perspective rather than a survey of the entire spectrum of thought.

The student of inverse theory must begin with a foundation in functional analysis. Parker builds on the work of pioneering inverse theoreticians such as George Backus and Freeman Gilbert, who in the 1960s built a sound mathematical basis for geophysical inversion, and placed inverse theory in terms of linear vector spaces (for example, Hilbert space). One of Parker's most valuable roles has been to interpret the sometimes opaque mathematics of these classical works and to express them in terms that are understandable to the numerate non-mathematician.

After a tutorial on vector spaces, Parker introduces linear inverse theory and then considers the effects of uncertain data (noise) on the resulting inverse models. It is here that formulation of the inverse problem in terms of Hilbert space is particularly convenient.

Parker provides a clear description

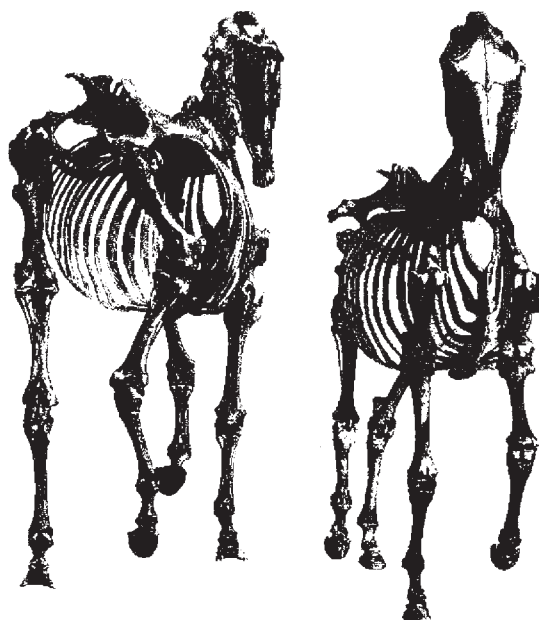
of linear resolution theory, and points out shortcomings in the classical Backus–Gilbert theory. A well-considered section on means of bounding linear functionals is followed naturally enough by ideal body theory, Parker's most important contribution to potential fields inversion (for example, gravitational and magnetic), and also by linear and quadratic programming, the methods by which such problems may be solved.

There is a brief discussion of a 'statistical theory' of inversion, but readers would do well to look elsewhere for a more detailed view of this approach. The final chapter is devoted to nonlinear inverse problems (most real-world problems are nonlinear) with particular emphasis on the mildly nonlinear magnetotelluric problem of finding the Earth's electrical-conductivity structure.

There is no general theory of nonlinear inversion; the usual approach is to reformulate the problem in linear terms around a starting model, and to solve it using an iterative process. Parker is a leading authority on this approach, and the final chapter is an excellent, although brief, introduction.

One might hope for an additional chapter in future editions touching on recent developments in nonlinear optimization theory, such as simulated annealing, genetic and evolutionary algorithms and the like. With this said, however, Parker's work is essential for any student of inverse theory and should be required reading for anyone in the field. □

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ANATOMICAL illustrations of the horse, taken from George Stubb's classic works of the eighteenth century. The engravings, which were based on detailed dissections, were done by Stubbs in 1766 and sold to subscribers as a set of 18. Reproduced from Bruce J. MacFadden's *Fossil Horses: Systematics, Paleobiology, and Evolution of the Family Equidae*, now published in paperback (for a review by Adrian Lister of the hardback edition see *Nature* 365, 118; 1993). Cambridge University Press, £14.95, \$29.95.

Chromosomal translocations in human cancer

T. H. Rabbitts

Chromosomal abnormalities in tumours were recognized at the end of the last century but their significance has only recently become clear. Distinct translocations in leukaemias and in solid tumours lead to the activation of proto-oncogene products or, more commonly, creation of tumour-specific fusion proteins. The proteins in both categories are often transcription factors and thus disruption of transcriptional control plays a major role in the aetiology of cancer. Fusion proteins formed after chromosomal translocations are common in a range of tumour types; these are unique tumour antigens and are therefore potential targets for therapy design.

CANCER is due to somatically acquired genetic changes, sometimes associated with inherited predisposing mutations such as those responsible for Wilms' tumour. The possibility that these chromosomal changes are responsible for neoplasia was proposed as early as 1900¹ but this was not generally accepted for some time, largely because cytogenetic techniques were not equal to the task of identifying consistent chromosomal changes. A combination of improved cytogenetics and molecular biology has now settled the issue. Recurring and highly consistent chromosomal aberrations have identified previously known and new proto-oncogenes at or spanning chromosomal breakpoints (Table 1). A variety of studies have shown that these genes are oncogenic, confirming the pivotal role of chromosomal aberrations in tumour development.

There are three main cytogenetic changes: deletions, translocations and inversions. The first often result in loss of a tumour-suppressor gene, and will not be discussed further. Translocations or inversions can be divided into those consistently found in certain tumour types (specific) and those observed only in the tumour from one patient (idiopathic). Molecular studies of breakpoints from specific aberrations have now reached a watershed. The breakpoints of many different and specific translocations and inversions (herein grouped as chromosomal translocations) have been cloned and some general principles have emerged from the study of the associated genes.

There are two principal consequences of translocations and inversions. Either the gene for a T-cell receptor (TCR) or an immunoglobulin protein comes to lie near a proto-oncogene, thereby activating it, or the breaks occur within a gene on each chromosome involved, creating a fusion gene encoding a chimeric protein. The genes involved often encode transcription factors, indicating that altered transcription plays a major part in tumorigenesis.

The first two chromosomal translocations studied at the molecular level illustrate these consequences (summarized in Fig. 1). The *c-MYC* gene translocation in Burkitt's lymphoma typifies the situation in which a proto-oncogene is juxtaposed to an immunoglobulin (or T-cell receptor) gene by chromosomal fusion with joining or diversity segments, thereby activating the oncogene. Immunoglobulin and T-cell receptor genes are frequently involved in chromosomal aberrations because they are naturally rearranged to generate active antigen-receptor genes. This process occasionally, in error, leads to an interchromosomal translocation or inversion, as in Burkitt's lymphoma. The fusion of the *BCR* and *c-ABL* genes on the Philadelphia chromosome in chronic myelogenous leukaemia, on the other hand, typifies the situation in which breakage on each chromosome occurs within introns of genes, producing fusion genes (Fig. 1b) and subsequently leading to expression of the fusion protein.

This work was followed by a spate of other examples, mainly involving immunoglobulin or T-cell receptor genes (Table 1a), although novel gene fusions were also found (Table 1b). Alteration or activation of transcription factors are key elements in most of these chromosomal abnormalities. Furthermore, it is now evident that the main consequence of chromosomal translocation in solid tumours is the formation of fusion proteins (Table 1c), suggesting that protein fusion will prove to be the main outcome of chromosomal translocations in general.

Proto-oncogene activation

Burkitt's lymphoma is a B-cell malignancy characterized by one of three chromosomal translocations. The most frequent is the translocation t(8; 14)(q24; q32), found in ~90 per cent of cases, juxtaposing *c-MYC* and immunoglobulin heavy-chain (H) genes (reviewed in ref. 2). The first clue that the immunoglobulin light-chain (L) genes might also be associated with this chromosomal translocation in 'variant' forms of African Burkitt's lymphoma was the mapping of κ L-chain gene (IgL κ) to chromosome 2, band p12 (ref. 3). Subsequently, variant forms of translocations in Burkitt's lymphoma were found to have breakpoints downstream of *c-MYC* and to involve breakpoints within IgL κ or λ genes in t(2; 8) and t(8; 22) respectively (reviewed in ref. 2). Although a great deal has been learnt about the *c-MYC* protein (see below), we still do not know why it is *c-MYC*, rather than other oncogenes, that is crucial for development of Burkitt's lymphoma. This is not simply explained by sequences near *c-MYC* facilitating translocation, because the chromosomal breakpoints are so disparate even though the mechanism of translocation is closely linked to immunoglobulin gene rearrangement. In addition, although the immunoglobulin or TCR-associated abnormalities do not generally alter the activated oncogene, there are often somatic mutations in the translocated *c-MYC* in both the non-coding exon 1 and within the coding region of exon 2 (reviewed in ref. 2). The latter cluster around residue 58, which is also mutated in several *v-myc* isolates, and these mutations may play a role in tumour progression.

The *c-MYC* protein has several functional domains, including a basic region for DNA binding and helix-loop-helix (b-HLH) and leucine zipper (ZIP) protein dimerization motifs. Activation of *c-MYC* by translocation compromises a transcriptional network involving at least three other factors, all of which also have b-HLH and ZIP domains (Fig. 2). *c-MYC* can dimerize with a protein called MAX^{4,5} which can bind to DNA and to proteins MAD and Mxi-1 (refs 6, 7) (MAX-MAX homodimers can also form). All these proteins are presumably in monomer-dimer equilibrium in the normal cell (Fig. 2). *c-MYC*-MAX dimers are transcriptionally active^{4,5}, whereas MAX-MAD or MAX-

TABLE 1 Chromosomal translocation breakpoints and genes

(a) Non-fusions/haematopoietic tumours

Type	Affected gene	Disease	Rearranging gene	Ref.
Basic-helix-loop-helix t(8; 14)(q24; q32) t(2; 8)(p12; q24) t(8; 22)(q24; q11) t(8; 14)(q24; q11) t(8; 12)(q24; q22)	c-MYC (8q24)	BL, BL-ALL	IgH, IgL	2
	c-MYC (8q24)	T-ALL	TCR- α	66
	c-MYC (8q24) BTG (12q22)	B-CLL/ALL	—	67
t(7; 19)(q35; p13) t(1; 14)(p32; q11) t(7; 9)(q35; q34)	LYL1 (19p13) TAL1/SCL (1p32) TAL2 (9q34)	T-ALL T-ALL T-ALL	TCR- β TCR- α TCR- β	68 20 20
LIM proteins t(11; 14)(p15; q11) t(11; 14)(p13; q11) t(7; 11)(q35; p13)	RBTN1/Ttg1 (11p15) RBTN2/Ttg2 (11p13)	T-ALL T-ALL	TCR- δ TCR- $\delta/\alpha/\beta$	22 22
Homeobox protein t(10; 14)(q24; q11) t(7; 10)(q35; q24)	HOX11 (10q24)	T-ALL	TCR- α/β	15–18
Zinc-finger protein t(3; 14)(q27; q32) t(3; 4)(q27; p11)	Laz3/BCL-6 (3q27) Laz3/BCL-6 (3q27)	NHL/DLCL NHL	IgH —	69, 70 69
Others t(11; 14)(q13; q32)	BCL-1/(PRAD-1) (11q13)	B-CLL and others	IgH	71
t(14; 18)(q32; 21) inv14 & t(14; 14)(q11q32) t(10; 14)(q24; q32) t(14; 19)(q32; q13.1) t(5; 14)(q31; q32) t(7; 9)(q34; q34.3) t(1; 7)(p34; q34) t(X; 14)(q28; q11)	BCL-2 (18q21) TCL-1 (14q32.1) Iyt-10 (10q24) BCL-3 (19q13.1) IL-3 (5q31) TAN1 (9q34.3) LCK (1p34) C6.1B (Xq28)	FL T-CLL B lymphoma B-CLL pre-B-ALL T-ALL T-ALL T-PLL	IgH, IgL TCR-C α IgH IgH IgH TCR- β TCR- β TCR- α	14 72 73 74–76 77, 78 79 80, 81 82, 83

(b) Gene fusions/haematopoietic tumours

Type	Affected gene	Protein domain	Fusion protein	Disease	Ref.
inv14 (q11; q32)	TCR- α (14q11) V α (14q32)	TCR-C α Ig V α	V α -TCR-C α	T/B-cell lymphoma	84, 85
t(9; 22)(q34; q11)	CABL (9q34) BCR (22q11)	tyrosine kinase serine kinase	serine + tyrosine kinase	CML/ALL	86
t(1; 19)(q23; p13.3)	PBX1 (1q23) E2A (19p13.3)	HD AD-b-HLH	AD + HD	pre-B-ALL	32, 33
t(17; 19)(q22; p13)	HLF (17q22) E2A (19p13)	bZIP AD-b-HLH	AD + bZIP	pro-B-ALL	42, 43
t(15; 17)(q21-q11-22)	PML (15q21) RARA (17q21)	Zinc-finger Retinoic acid receptor- α	Zinc-finger + RAR DNA and ligand binding	APL	39
t(11; 17)(q23; q21.1)	PLZF (11q23) RARA (17q21)	Zinc-finger Retinoic acid receptor- α	Zn-finger + RAR DNA and ligand binding	APL	40
t(4; 11)(q21; q23)	MLL (11q23) AF4 (4q21)	A-T hook/Zn-finger Ser-Pro rich	A-T hook + Ser-pro	ALL/preB-ALL/ANLL	44, 45
t(9; 11)(q21; q23)	MLL (11q23) AF9/MLLT3 (9p22)	A-T hook/Zn-finger Ser-Pro rich	A-T hook + (Ser-Pro)	ALL/preB-ALL/ANLL	87, 88
t(11; 19)(q23; p13)	MLL (11q23) ENL (19p13)	A-T hook/Zn-finger Ser-Pro rich	A-T hook + Ser-Pro	pre-B-ALL/T-ALL/ANLL	46, 89
t(X; 11)(q13; q23)	MLL (11q23) AFX1 (Xq13)	A-T hook/Zn-finger (Ser-Pro rich)	A-T hook + (Ser-Pro)	T-ALL	47
t(1; 11)(p32; q23)	MLL (11q23) AF1P (1p32)	A-T hook/Zn-finger Eps-15 homologue	A-T hook + ?	ALL	90
t(6; 11)(q27; q23)	MLL (11q23) AF6 (6q27)	A-T hook/Zn-finger myosin homologue	A-T hook + ?	ALL	91
t(11; 17)(q23; q21)	MLL (11q23) AF17 (17q21)	A-T hook/Zn-finger Cys-rich/leucine zipper	A-T hook + leucine zipper	AML	92
t(8; 21)(q22; q22)	AML1/CBF α (21q22) ETO/MTG8 (8q22)	DNA binding/runt homology Zn-finger	DNA binding + Zn-fingers	AML	34
t(3; 21)(q26; q22)	AML1 (21q22) EVI-1 (3q26)	DNA binding Zn-finger	DNA binding + Zn-fingers	CML	93
t(3; 21)(q26; q22)	AML1 (21q22) EAP (3q26)	DNA binding Sn protein	DNA binding + out-of-frame EAP	Myelodysplasia	94
t(16; 21)(p11; q22)	FUS (16p11)	Gln-Ser-Tyr/Gly-rich/RNA binding	Gln-Ser-Tyr + DNA binding	Myeloid	61
	ERG (21q22)	Ets-like DNA binding			
t(6; 9)(p23; q34)	DEK (6p23) CAN (9q34)	? ZIP	? + ZIP	AML	95

TABLE 1—continued

(b) Gene fusions/haematopoietic tumours—continued

Type	Affected gene	Protein domain	Fusion protein	Disease	Ref.
9; 9?	<i>SET</i> (9q34)	?	? + ZIP	AUL	96
	<i>CAN</i> (9p34)	ZIP			
t(4; 16)(q26; p13)	<i>IL-2</i> (4q26)	IL2	IL-2/TM	T-lymphoma	35
	<i>BCM</i> (16p13.1)	?/TM domain			
inv(2; 2)(p13; p11.2-14)	<i>REL</i> (2p13)	DNA binding-activator	DNA binding + ?	NHL	97
	<i>NRG</i> (2p11.2-14)	not known			
inv(16)(p13q22)	Myosin MYH11 (16p13)		DNA binding?	AML	98
	<i>CBF-β</i> (16q22)				
t(5; 12)(q33; p13)	<i>PDGF-β</i> (5q33)	Receptor kinase	Kinase + DNA binding	CMML	36
	<i>TEL</i> (12p13)	Ets-like DNA binding			
t(2; 5)(2p23; q35)	<i>NPM</i> (5q35)	Nucleolar phosphoprotein	N terminus NPM + kinase	NHL	37
	<i>ALK</i> (2p23)	Tyrosine kinase			

(c) Gene fusions/solid tumours

Type	Affected gene	Protein domain	Fusion protein	Disease	Ref.
inv10(q11.2; q21)	<i>RET</i> (10q11.2)	tyrosine kinase	Unk + tyrosine kinase	Papillary thyroid carcinoma	48
	<i>D10S170</i> (q21)	uncharacterized			
t(11; 22)(q24; q12)	<i>FLI1</i> (11q24)	Ets-like DNA binding	Gln-Ser-Tyr + DNA binding	Ewing's sarcoma	49
	<i>EWS</i> (22q12)	Gln-Ser-Tyr/Gly-rich/RNA binding			
t(21; 22)(?; q12)	<i>ERG</i> (21q22)	Ets-like DNA binding	Gln-Ser-Tyr + DNA binding	Ewing's sarcoma	58
	<i>EWS</i> (22q12)	Gln-Ser-Tyr/Gly-rich/RNA binding			
t(12; 22)(q13; q12)	<i>ATF1</i> (12q13)	bZIP	Gln-Ser-Tyr + bZIP	Melanoma of soft parts	59
	<i>EWS</i> (22q12)	Gln-Ser-Tyr/Gly-rich/RNA binding			
t(12; 16)(q13; p11)	<i>CHOP</i> (12q13)	(DNA binding?)/ZIP	Gln-Ser-Tyr	Liposarcoma	50, 51
	<i>FUS</i> (16p11)	Gln-Ser-Tyr/Gly-rich/RNA binding	+ (DNA binding?)/ZIP		
t(2; 13)(q35; q14)	<i>PAX3</i> (2q35)	Paired box/homeodomain	PB/HD + DNA binding	Rhabdomyosarcoma	55
	<i>FKHR</i> (13q14)	Forkhead domain			
t(X; 18)(p11.2; q11.2)	<i>SYT</i> (18q11.2)	None identified		Synovial sarcoma	54
	<i>SSX</i> (Xp11.2)	None identified			

The main protein motifs or structural elements are usually deduced from cDNA sequence. In *b*, the motifs indicated in the putative fusion proteins are those likely to be the important molecule in tumour pathogenesis. In most cases this is an assumption which is not yet confirmed by functional data. Note that the consequence of gene fusion between MLL and AF9 is that the extent of AF9 protein fused to MLL in translocation t(9;11) is variable and in one fusion the AF9 region is not Ser-Pro-rich. This variability is indicated by parentheses. In addition, only a small amount of AFX1 sequence is yet available and, although this indicates a Ser-Pro-rich sequence, this remains to be verified. Abbreviations used: Known and putative DNA binding and/or protein dimerization motifs—PB, paired box; HD, homeodomain; bZIP, basic region leucine zipper; b-HLH, basic helix-loop-helix motif; ZIP, leucine zipper motif; LIM, cysteine-rich motif. Disease nomenclature: BL, Burkitt's lymphoma; FL, follicular lymphoma; AL, acute leukaemia; CL, chronic leukaemia; ALL, acute lymphocytic leukaemia (T- or B-cell); CLL, chronic lymphocytic leukaemia (T- or B-cell); PLL, prolymphocytic leukaemia; CML, chronic myelogenous leukaemia; AML, acute myelogenous leukaemia; APL, acute promyelocytic leukaemia; AUL, acute undifferentiated leukaemia; NHL, non-Hodgkin's lymphoma; CMML, chronic myelomonocytic leukaemia; DLCL, diffuse large-cell lymphoma. Other abbreviations: AD, transcriptional activation domain; TM, TM sequence; RARA, retinoic acid receptor- α receptor; IL, interleukin; Ph, Philadelphia chromosome; Unk, unknown.

Mxi-1 dimers are not. After chromosomal translocation, this equilibrium is disrupted by incorrect expression of c-MYC. As the c-MYC-MAX dimer is necessary for oncogenic activation⁸, excess c-MYC expression stemming from chromosomal translocation would cause a shift in equilibrium (Fig. 2) and presumably result in transcription of downstream target genes, leading to oncogenesis.

Many other translocations and inversions result in gene activation. Most genes involved are transcription factors but there are exceptions. In the chronic forms of leukaemia and lymphoma, various genes are found near chromosomal breakpoints (Table 1a), implying that development of chronic disease can occur in various ways. The *BCL2* protein has received much attention because it protects B and T cells from apoptosis in mice transgenic for *bcl2* expression^{9, 11}. Transgenic mice develop B-cell hyperplasia and eventually B-cell malignancy^{10, 11}. *Bcl2* protein apparently mediates a block to apoptosis by regulating antioxidant pathways and decreasing production of reactive oxygen species^{12, 13}. These observations provide an explanation for the human lymphoid cancer follicular lymphoma, which is a chronic disease occurring over many years, invariably associated with the translocation t(14; 18)(q32; 21) juxtaposing the *IgH*-joining region with the *BCL2* gene from 18q21, thereby constitutively activating *BCL2* (reviewed in ref. 14). Similar situations are doubtless applicable to other chronic tumours, such as T-cell

chronic leukaemia (T-CLL), which is associated with inversion or translocation of the long arm chromosome 14. T-CLL can also exist for many years before overt T-cell malignancy. The putative proto-oncogene at the 14q32.1 region has yet to be cloned but promises to be of great interest.

Acute T-cell leukaemias

Continuing the theme of proto-oncogenes activated by chromosomal translocations with antigen-receptor genes, T-cell acute leukaemias (T-ALL) have a number of different recurring translocations (Table 1a). Most of these involve putative transcription factors, are involved in differentiation and are not normally expressed in T cells. An example of this is the *HOX11* gene, located on chromosome 10, band q24, which is activated by translocations t(10; 14)(q24; q11) and t(7; 10)(q35; q24) in T-ALL (refs 15–18). This gene encodes a homeodomain protein that can bind DNA and transactivate transcription¹⁹. As it is not expressed in normal T cells, the *HOX11* protein may well activate target genes in T cells with a 10q24 translocation, contributing to T-ALL.

Several DNA-binding proteins activated by chromosomal translocation in T-ALL contain a b-HLH motif (Table 1a). The *TALI/SCL* gene is an important example. This gene is either involved in t(1; 14) translocation or, in up to 25% of childhood T-ALL cases, has undergone a submicroscopic deletion on the

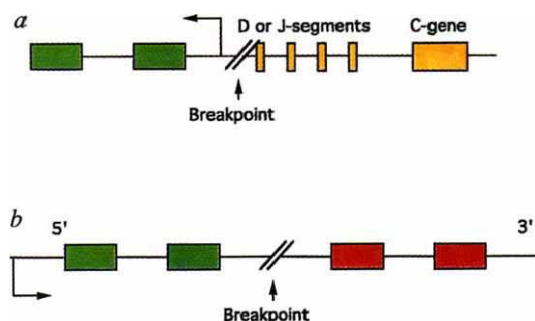


FIG. 1 Major consequences of chromosomal translocations and inversions. *a*, Gene activation by joining to immunoglobulin or T-cell receptor genes. The chromosomal translocation breakpoints generally occur at diversity (D) or joining region (J) segments (the normal site of variable-region gene joining) which are depicted as yellow boxes upstream of the constant (C) region gene segment. The activated oncogene (green exons) lies on the other side of the breakpoint. The transcriptional promoter of the oncogene (bent arrow) may remain intact after the translocation as shown. *b*, Gene fusion. In the example shown, exons of two hypothetical genes (depicted in green and red) have become joined as a result of a translocation or inversion. Transcription of the resultant chimaeric gene would run from the promoter of the 'green' gene to the polyadenylation site of the 'red' gene producing an mRNA encoding a fusion protein comprising parts of both green and red proteins.

5' side of the gene (reviewed in ref. 20). The protein encoded by *TAL1* can dimerize with the protein E47 (ref. 21), forming a DNA-binding complex. The chromosomal translocation probably causes ectopic *TAL1* production, activating a specific set of target genes which are normally silent in T cells. Similar models may apply to other T-ALL-associated proteins activated by chromosomal translocations, such as the *RBTN/Ttg* proteins (reviewed in ref. 22). These are produced after translocations *t*(11; 14)(p13; q11) or *t*(11; 14)(p15; q11) and encode proteins with a LIM motif²³. The cysteine-rich LIM motif has a zinc-finger-like structure²⁴ and, as previously suggested²⁵, the LIM domain can function in protein-protein interaction as *RBTN2* can bind to the b-HLH protein *TAL1* (refs 26, 27). Although *RBTN2* induces T-cell tumours in transgenic mice, it is essential in normal mouse haematopoiesis for erythroid differentiation²⁸. It therefore seems likely that transcription of target genes, normally not involved in T cells, are affected after *RBTN2* activation by chromosomal translocation. Whatever its mode of action, the ectopic expression of LIM-containing proteins as a result of translocation in T cells may have similar effects to *HOX11* and *TAL1* expression.

Chimaeric proteins

Although chromosomal aberrations might be thought to activate most proto-oncogenes by juxtaposing them to genes that normally undergo rearrangement, the study of increasing numbers of abnormalities (Table 1*b, c*) indicates that gene fusion, resulting in the synthesis of chimaeric proteins, is the main repercussion.

The first documented example came from the cloning of the Philadelphia chromosome breakpoint²⁹. In this abnormality, the cellular homologue of the Abelson transforming gene, *c-ABL* (9q34), is fused with the *BCR* gene (22q11)^{30,31}. This fusion creates a tumour-specific marker and the functional consequence of the *BCR-ABL* fusion is increased tyrosine kinase activity.

These studies of the *BCR-ABL* fusion have paved the way for many more descriptions of fusion proteins, often involving transcription factors (Table 1*b, c*): for example proteins that bind DNA, like *PBX* in the pre-B-ALL-associated *t*(1; 19)^{32,33} or *ETO/MG8* in the AML-associated *t*(8; 21) (reviewed in ref. 34). Nonetheless, although gene fusion is the predominant phenomenon, transcription factors are not the only proteins

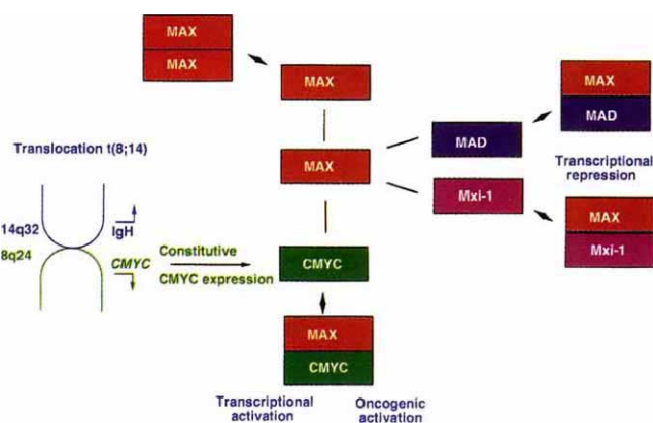


FIG. 2 The c-MYC-MAX transcription network in Burkitt's lymphoma. The c-MYC gene is activated by the chromosomal translocations *t*(8; 14) (shown), *t*(8; 22) and *t*(2; 8), all involving an immunoglobulin locus. c-MYC can dimerize with a protein called MAX, which can in turn dimerize with itself or with factors called MAD or Mxi-1. Constitutive expression of c-MYC caused by chromosomal translocations shifts the equilibrium towards c-MYC-MAX dimers, activating transcription.

involved in fusions after translocation, as for example the fusion of the interleukin-2 (*IL-2*) gene with what seems to be a transmembrane domain of a protein encoded by a gene designated BCM in a T-cell lymphoma³⁵. An interesting twist is the fusion of kinase domains to nuclear proteins, shown recently for the platelet-derived growth factor (*PDGF-β*) receptor kinase fused to an Ets-family transcription factor *Tel* in chronic myelomonocytic leukaemia³⁶ and a new tyrosine kinase, *ALK*, fused to a nucleolar protein, *NPM*, in non-Hodgkin's lymphoma³⁷. These putative kinase-transcription factors created by translocations may have natural counterparts in the LIM family, such as the kinase-LIM protein *LIMK*³⁸.

An important example of transcription factor fusion occurs after the translocation *t*(15; 17)(q21-q11-22) in acute promyelocytic leukaemia (APL). This translocation involves the gene for the retinoic acid- α receptor (*RARA*) (reviewed in ref. 39) and patients respond well to treatment with all-*trans* retinoic acid. The translocation breakpoint consistently occurs within the *RARA* gene and within a gene designated *PML*. The *RARA* is a nuclear receptor which binds the retinoic acid ligand and to

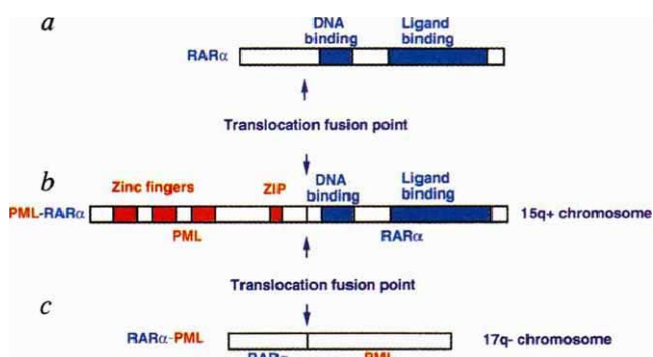


FIG. 3 Fusion of retinoic acid receptor- α and PML in acute promyelocytic leukaemia by translocation *t*(15; 17). *a*, *RARα* protein. The DNA-binding and ligand-binding domains are shown in blue. *b*, *PML-RARα* fusion protein. Both the DNA- and ligand-binding domains of *RARA* are retained on the derivative 15q⁺ chromosome. This fusion also includes presumptive DNA-binding zinc-fingers of *PML* and a leucine zipper region (ZIP) (all shown in red). *c*, *RARα-PML* fusion protein. The protein encoded by the derivative 17q⁻ chromosome lacks the striking motifs of the reciprocal product.

FIG. 4 The fusion of E2A and PBX in the pre-B ALL translocation t(1; 19). The transcriptional activation domain of E2A, but not the basic-helix-loop-helix domain, is fused to part of PBX1, including the DNA-binding homeodomain (blue box), giving the activation domain a new potential DNA-binding specificity.

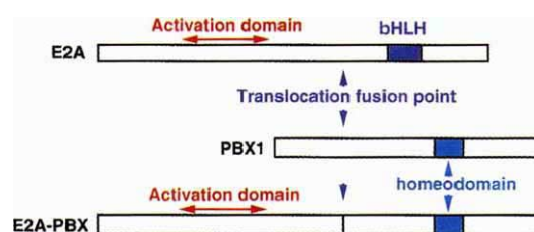


FIG. 5 Chromosomal abnormalities in acute leukaemias involving the *MLL* gene on chromosome 11, band q23. *a*, Chromosomal abnormalities. The *MLL* gene lies near the tip of the long arm of chromosome 11 and is split in the translocations and interstitial deletions shown. The breakage regularly occurs upstream of the region coding for the zinc-fingers (exons 8 to 12) of the protein. The derivative chromosome retaining the chromosome 11 centromere is probably responsible for leukaemogenesis⁶⁵, and the protein involved therefore lacks the zinc-fingers. *b*, Consequences for the *MLL* protein after t(11; 19). In t(11; 19), the N-terminal part of *MLL* (top line) is fused to most of *ENL* from chromosome 19 (middle line) to create the protein illustrated on the bottom line. The *MLL* protein has four regions homologous to other proteins, an A-T hook region, a region homologous to DNA methyltransferase (MT), zinc-fingers (yellow) and a region with homology to the *Drosophila* trithorax gene (blue). Middle line shows the *ENL* protein which is serine- and proline-rich; the major break lies near the N terminus so that virtually the whole *ENL* protein is fused with *MLL*.

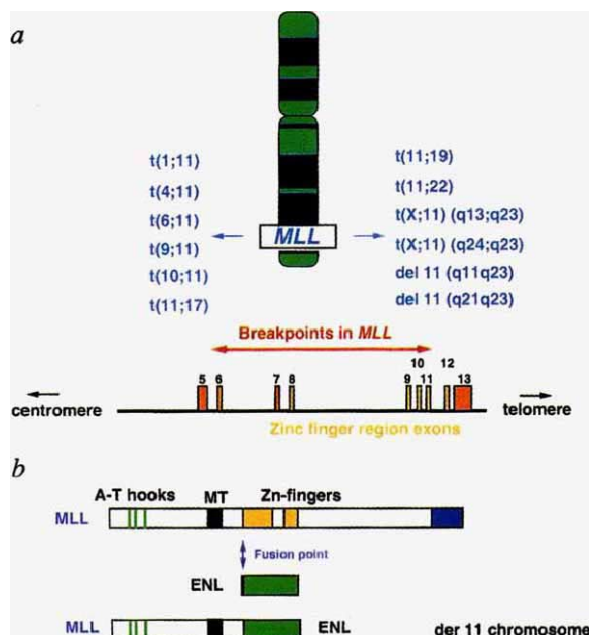
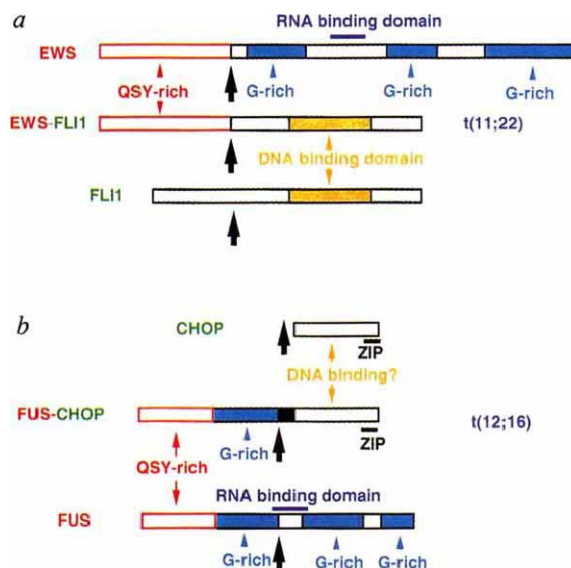


FIG. 6 Chimaerism of transcription factors after chromosomal translocation in Ewing's sarcoma and malignant liposarcoma. These sarcomas consistently carry translocations t(11; 22) or t(12; 16), respectively; both result in gene fusions analogous to those found in leukaemias. The large black inverted arrows indicate translocation fusion points. *a*, EWS-FLI1 fusion in t(11; 22) of Ewing's sarcoma. The t(11; 22) consistently breaks the *EWS* gene on chromosome 22 and the *FLI1* gene on chromosome 11. *FLI1* contains an Ets-like DNA-binding domain which forms the C-terminal region of the fusion protein. *EWS* is a glycine-rich protein (blue) together with a glutamine-serine-tyrosine-rich (QSY) N-terminal portion (red box) and a domain homologous to RNA-binding proteins. In the example shown (type 1 fusion⁴⁹), the QSY-rich portion of *EWS* is fused with the DNA-binding domain of *FLI1*. *b*, FUS-CHOP fusion by t(12; 16) in liposarcoma. The t(12; 16) involves the *CHOP* gene on chromosome 12 and a gene designated *FUS* on chromosome 16. The *CHOP* gene encodes a leucine zipper (ZIP) protein. *FUS* encodes a protein with features similar to those of *EWS*. The breakage in *FUS*, like that in *EWS*, excludes the RNA-binding domain from the FUS-CHOP fusion but includes the QSY-rich stretch and one of the glycine-rich stretches. The breakage in *CHOP* leaves the coding sequences intact and includes a segment translated from a normally non-coding exon from *CHOP*.



DNA through a zinc-finger region (Fig. 3a), thereby presumably activating a set of target genes. Similarly, the PML protein is a DNA-binding zinc-finger protein with a potential leucine zipper motif. The more important of the two fusion proteins created by the translocation seems to be the PML-RARA fusion because this has the important functional domains of both proteins (Fig. 3b, c).

What is the role of the PML-RARA fusion protein? Although it may retain the capacity to dimerize and bind DNA, the situation is complicated by its ability to bind retinoic acid. It may therefore affect the transcription of genes influenced by either parent in a retinoic-acid-dependent manner. A variant translocation

t(11; 17)(q23;q21)⁴⁰ fuses RARA to another putative transcription factor designated PLZF (for promyelocytic leukaemia zinc-finger) producing a protein like PML-RARA, in which part of RARA is fused to the N-terminal part of PLZF (including two of its nine zinc-fingers)⁴⁰. This rare translocation lends strength to the view that a consequence of translocation in APL is conferring new DNA-binding specificity to RARA.

An analogous situation occurs in the pre-B-cell acute leukaemias (pre-B-ALL) with the translocation t(1; 19), which fuses the N-terminal part of the transcription factor E2A, carrying a transactivation domain, to the DNA-binding homeodomain of the transcription factor PBX^{32,33}, replacing the b-HLH

region of E2A (Fig. 4). The fusion protein can strongly activate transcription⁴¹ whereas PBX cannot, at least in reporter systems. Target genes that bind the PBX homeodomain in the fusion protein may thus be activated and initiate tumour development.

The rare t(17; 19) in B-ALL also involves E2A fusion, but in this case with a protein called HLF which has a basic-leucine-zipper domain (bZIP). The DNA-binding plus protein-dimerization domains of HLF are fused to a part of E2A that again includes the transcriptional activation domain^{42,43}. HLF can bind DNA and activate transcription alone, but is not normally expressed in lymphoid cells^{42,43}. The chromosomal translocation places the fusion gene under the control of the E2A promoter, which is active in lymphoid cells, presumably stimulating target genes normally controlled by HLF.

In childhood acute leukaemias, the 11q23 region is frequently affected by a remarkable variety of chromosomal translocations and deletions (Fig. 5), giving rise to tumours with a range of haematopoietic phenotypes. In all cases, the *MLL* genes (also called *ALL-1*, *HRX*, *HTRX*)⁴⁴⁻⁴⁶ is broken within an extremely small region, about half-way through the coding region of the gene (Fig. 5). The result is a series of acute leukaemia-specific chimaeric proteins, thought to be crucial to tumour development and bearing the N terminus of *MLL*. The *MLL* gene encodes a protein with several sequence motifs, including N-terminal A-T hooks, a region homologous to mammalian methyltransferases (MT), a region with zinc-fingers and a C-terminal region homologous to the *Drosophila* trithorax gene^{45,46} (Fig. 5b). In each translocation, the zinc-finger region of the *MLL* is lost but the A-T hook is not, and the MT homology regions are lost from the crucial derivative-11 chromosome (Fig. 5b). In t(11; 19), *ENL* is fused to *MLL* after breakage of the *ENL* gene very close to the N-terminal region⁴⁶, yielding an *MLL-ENL* fusion with A-T hook and MT regions linked to a protein segment which is rich in serine and proline. Tumours of differing phenotype can have similar or identical chimaeric proteins⁴⁷, so the tumour phenotype is not dictated by the translocation alone. A-T hooks are thought to bind the minor groove of DNA and may allow other transcription factors to gain access. The role of the MT-homologous domain is not clear but may allow the *MLL* fusion proteins to modify the methylation status of DNA and thereby affect transcription.

Despite the variety of chromosomal changes affecting 11q23, two consistent features of the resulting fusion proteins are apparent. First, the *MLL* zinc-finger region is lost, probably abolishing specific DNA binding. Second, the A-T hook and MT domain are retained, possibly allowing nonspecific DNA binding and alteration of methylation. It may well be that the fusion partner is not as important as truncation of *MLL*. Thus although these multidomain fusion proteins are of interest, it will be necessary to establish *in vivo* model systems mimicking the consequences of different *MLL* fusions before the full effects can be understood.

Chimaeric transcription factors

It has become evident that protein fusion in leukaemia was at least as common as proto-oncogene activation by juxtaposition with an antigen-receptor gene. The latter are particularly prone to participate in chromosomal translocations because they normally undergo rearrangement in lymphoid development, but this process seems restricted to the immune system. A corollary is therefore that most chromosomal translocations are likely to result in protein fusion in other tumours.

Initial studies with solid tumours have confirmed this idea and have identified chimaeric transcription factors formed by chromosomal abnormalities. The first abnormality to be studied in solid tumours was an inversion of chromosome 10 found in papillary thyroid carcinoma, fusing of the *RET* gene (10q11) with a uncharacterized gene on the other side of the inversion⁴⁸.

Investigation of solid-tumour translocations has concentrated on the sarcomas, whose cytogenetics have been well studied. The structural consequences of four of these translocations have been

solved (Table 1c) and result in chimaeric proteins in Ewing's sarcoma, liposarcoma, rhabdomyosarcoma and synovial sarcoma⁴⁹⁻⁵⁴.

In Ewing's sarcoma, the t(11; 22) fuses a region rich in glutamine, serine and tyrosine residues from a gene called *EWS* to the Ets-like DNA binding domain of the *FLI1* gene⁴⁹ (previously known as an insertion site for the Friend leukaemia virus in virally induced erythroleukaemias⁵⁵) (Fig. 6a). The normal *EWS* protein also has three glycine-rich segments and a domain homologous to RNA-binding proteins⁴⁹. The breakpoints in the two genes are remarkably consistent in that the N-terminal part of *EWS* (including the glutamine-serine-tyrosine-rich segment) is generally fused to the DNA-binding domain of *FLI1* (ref. 56); DNA binding by the *FLI1* domain and transcriptional activation by fusion protein are crucial components in tumorigenesis⁵⁷. In addition, a new translocation has been found in a Ewing's sarcoma, between *EWS* and a gene called *ERG* (from chromosome 21) which also encodes an Ets-like DNA-binding domain related to *FLI1* (refs 56, 58). The putative *EWS-ERG* fusion protein presumably acts in a similar way to *EWS-FLI1*. The *EWS* gene also plays a role in other tumours, as a translocation t(12; 22) in malignant melanoma fuses *EWS* with the *ATF-1* gene⁵⁹, resulting in a protein again resembling *EWS-FLI1* and containing the glutamine-serine-tyrosine-rich segment of *EWS* fused with a DNA-binding bZIP segment of the *ATF-1* protein. Thus, the DNA-binding and dimerization capacities of *ATF-1* in the fusion protein probably lead to activation of *ATF-1* targets in melanoma. An interesting point is that certain oncogenes are activated in different tumours, as illustrated by the fusion of *RET* by chromosomal inversion in papillary thyroid carcinoma and the inherited mutations of the gene in multiple endocrine neoplasia type-2A⁶⁰ or the different translocations of the Ets-like protein *ERG* in Ewing's sarcoma⁵⁸ and in myeloid leukaemia⁶¹.

The translocation t(12; 16) in malignant liposarcoma produces a very similar product by fusing a gene on chromosome 16 called *FUS* or *TLS*^{50,51} and the gene for a dominant inhibitor of transcription, *CHOP*^{50,51}. Like the *EWS* protein, the *FUS* product contains a glutamine-serine-tyrosine-rich segment, three glycine-rich stretches^{50,51} and an RNA-binding domain⁵⁰ (Fig. 6b). The fusion point in *FUS* resembles that in *EWS* as the glutamine-serine-tyrosine-rich segment and one of the three glycine-rich sequences is fused to the entire *CHOP* coding region^{50,51} (Fig. 6b). The natural *CHOP* product possesses a leucine zipper dimerization domain, and the protein can bind to CATA-box-binding (C/EBP-like) proteins, blocking their interaction with DNA⁶². This leucine zipper is retained after the translocation. The *FUS* domain in the fusion protein provides a transcriptional activation domain⁶³ to a presumptive DNA-binding activity of *CHOP*, as in the *EWS* fusion. The t(X; 18) in synovial sarcoma also results in a fusion between two novel genes *SYT* and *SSX*⁵⁴. Although there is currently no evidence that this results in a fusion transcription factor, *SYT* is glutamine-, proline- and glycine-rich and may be a transcriptional activation domain in the fusion protein like *FUS* in the *FUS-CHOP* fusion⁶³.

Another example of a transcription factor activated by translocation is provided by the translocation t(2; 13) in the solid tumour rhabdomyosarcoma, which fuses the paired-box homeodomain region of the *PAX3* gene with a forkhead-domain gene *FKHR*⁵³. Like other *PAX* developmental control genes, *PAX3* encodes a protein which is a transcription factor with DNA-binding domains (paired box and homeodomain) and which has a role in development by activating specific target genes. After the translocation t(2; 13), a fusion protein is made with the DNA-binding domain of *PAX3*, a truncated *FKHR* DNA-binding domain and the C-terminal region of *FKHR*⁵³. This fusion is interesting because various normal *PAX* genes can transform 3T3 cells⁶⁴, supporting the view, drawn from studies of chromosomal translocations, that a wide variety of DNA-

binding factors can be key components in tumour development if inappropriately presented.

Conclusions

The chromosomal translocations studied in many haematological tumours and in some solid tumours have established three general principles. First, consistent and specific translocations and inversions are involved in tumour aetiology, activating proto-oncogenes at or near the chromosomal breakpoints in a wide range of tumours. Second, although many haematological translocations or inversions involve immunoglobulin and T-cell receptor genes, which are normally rearranged in lymphoid development, they usually result in the formation of a tumour-specific fusion protein. Other fusion proteins are also crucial in solid tumours, which comprise about 98% of cancer mortalities. Third, and most frequently, transcription factors are affected, presumably resulting in the activation of downstream target genes.

The cloning of further breakpoints is unlikely to reveal new principles governing the contribution of translocations or inversions to cancer, although it will uncover new genes and gene fusions of intrinsic interest. Indeed, this approach has led to the discovery of new transcription factors. In addition, the identification of new oncogenes will continue to be important as potential targets for therapy. The target genes controlled by the various transcription factors affected by translocations are also largely unknown and will be a focus of future experiments, allowing any interrelationship of the transcription networks to be assessed in tumorigenesis.

Do these observations have practical value in medicine? The fusion proteins generated by translocations are true tumour-specific antigens, and therefore potential targets for therapy. Unfortunately, they are generally nuclear proteins, and invariably intracellular ones, necessitating the introduction of therapeutic molecules into the tumour cell. Therapeutic strategies could be applied to interfere with any step between transcription of the oncogene, RNA processing and translation, and function of the protein. Such gene therapy approaches to cancer treatment may have advantages as conventional methods, such as chemotherapy, often result in the appearance of resistant cells. If the presence of fusion protein is necessary for the persistence of tumour, the outgrowth of resistant cells is less likely.

The chimaeric RNA in tumours will also provide an important target for polymerase chain reaction (PCR)-based detection of residual tumour cells after treatment and may also assist in early detection of tumours. This would be simplest in haematological tumours, where material is readily accessible for sampling. As our knowledge of tumour biology increases, however, it may be possible to use these molecular biological observations to combat all types of cancer.

Note added in proof: The *FUS* gene, first discovered in the liposarcoma fusion^{50,51}, has been shown to fuse with the Ets-like protein *ERG* in some AML patients with t(16;21) (refs 99, 100). □

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Oligosaccharide ligands for NKR-P1 protein activate NK cells and cytotoxicity

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A diversity of high-affinity oligosaccharide ligands are identified for NKR-P1, a membrane protein on natural killer (NK) cells which contains an extracellular Ca^{2+} -dependent lectin domain. Interactions of such oligosaccharides on the target cell surface with NKR-P1 on the killer cell surface are crucial both for target cell recognition and for delivery of stimulatory or inhibitory signals linked to the NK cytolytic machinery. NK-resistant tumour cells are rendered susceptible by preincubation with liposomes expressing NKR-P1 ligands, suggesting that purging of tumour or virally infected cells *in vivo* may be a therapeutic possibility.

UNDERSTANDING the biological roles of the diverse oligosaccharides of glycoproteins, proteoglycans and glycolipids has been a major challenge in cell biology. Work with monoclonal antibodies has shown that there are great changes in the display of oligosaccharides at the surface of cells during embryonic development, differentiation, and in malignancy^{1,2}. This suggested that such oligosaccharides (among which are the major blood group antigens and related sequences) may be important as ligands in macromolecular interactions that determine the way cells migrate or respond to various microenvironments^{1,3,4}. Prospects for rapid progress in this area have increased with the growth of our knowledge on proteins that contain carbohydrate-recognition domains (CRDs). Prominent among these are the calcium-dependent, C-type, lectin family⁵, several members of which bind carbohydrates⁶. In this family are the selectins⁷⁻⁹, which have crucial roles in leukocyte recruitment in inflammation and almost certainly play a role in the blood-borne spread of tumour cells.

Proteins of the C-type lectin family have been described on natural killer (NK) cells of rat, mouse and human^{10,11}. This is the population of lymphocytes that displays innate immunity to destroy certain tumours and virally infected cells, and which may play a role in control of cell numbers during haematopoiesis, and in transplantation reactions (graft-versus-host disease)¹². The lectin-like molecules on NK cells are dimeric type II transmembrane proteins each containing an extracellular C-type lectin-like domain, a stalk region and a cytoplasmic tail with tyrosine and serine residues which are potential phosphorylation sites. The first of these proteins to have been cloned and sequenced is NKR-P1 of rat¹³, several isoforms of which occur simultaneously on NK cells of the rat and mouse^{14,15}. Antibodies bound to NKR-P1 on the surface of NK cells can induce antibody-dependent cytotoxicity of FcR^+ target cells¹⁶, and crosslinking NKR-P1 with antibodies stimulates phosphoinositide turnover and mobilization of intracellular calcium¹⁷. For these reasons there has been considerable interest in identifying the ligands for this protein and elucidating its involvement in the cascade of events that results in the killing of target cells.

We have recently expressed in *Escherichia coli* a recombinant soluble form of NKR-P1 (referred to here as sNKR-P1) corresponding to the dimeric extracellular portion of NKR-P1, and have shown that it is a calcium-dependent carbohydrate-binding protein with a monosaccharide-binding pattern different from those described for several other C-type lectins, and an unusually tight association with calcium¹⁸. The protein has a higher affinity of binding to β -N-acetyl-D-galactosamine than to β -N-acetyl-D-glucosamine, and a lower affinity towards α -L-fucose, with half-maximum inhibitory concentration (IC_{50}) values of 0.6×10^{-7} M, 2×10^{-7} M and 2×10^{-6} M, respectively. Here we identify several extremely potent saccharide ligands for NKR-P1, some with IC_{50} values in the range 10^{-9} – 10^{-12} M. These include oligosaccharide sequences of the blood group family, the ganglio family and glycosaminoglycans. In addition, we identify such ligands on NK-susceptible tumour cells and provide evidence that interactions of oligosaccharide ligands on the target cell surface with NKR-P1 (or closely related proteins) at the NK cell surface are intimately involved in mechanisms of NK cell triggering that lead to cytolysis of target cells.

High-affinity saccharide ligands for NKR-P1

We have used as a reference compound the lipid-linked trisaccharide, DLNN (see Table 1 for definitions), with a terminal *N*-acetylglucosamine (Table 1), known from our earlier study¹⁸ to be bound by sNKR-P1. In chromatogram-binding experiments sNKR-P1 was found to bind to the LNT sequence (galactose β 1-3-linked to the *N*-acetylglucosamine of the DLNN) but not to the LNNT sequence (galactose β 1-4-linked) (Fig. 1a, lanes 1', 2' and 3'). However, in the presence of α 1-3-linked fucose on the *N*-acetylglucosamine of LNNT (Le^x antigen, LX5), there was binding (lane 2'). For LNT backbone there was some enhancement of binding in the presence of α 1-4-linked fucose (Le^a antigen, LA5 in lane 3'). The blood group H and Le^b sequences containing fucose α 1-2-linked to galactose on the LNT and LA5 sequences as in H5 (lane 1') and LB6 (lane 3') were also bound. However, the blood group A monosaccharide, α -N-acetyl-D-galactosamine, masks the binding sites on the H and Le^b sequences as in A6 and A7, respectively (results not shown). In additional experiments (not shown) the glycolipid globoside, with the oligosaccharide sequence $\text{GalNAc}\beta$ 1-

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TABLE 1 Monosaccharide and oligosaccharide sequences investigated in the present studies in the free or lipid-linked form

Designation	Abbvn	Structure	Designation	Abbvn	Structure
Neutral type 1			GD ₃	GD3	NeuAcα2-8NeuAcα2-3Galβ1-4Glc
Lacto- <i>N</i> -tetra	LNT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	GD ₂	GD2	GalNAcβ1-4Galβ1-4Glc
H penta	H5	Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,2} Fucα			NeuAcα2-8NeuAcα _{2,3}
Le ^a penta	LA5	Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,4} Fucα	GD _{1a}	GD1A	NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glc _{2,3} NeuAcα
Le ^b hexa	LB6	Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,2} _{1,4} Fucα Fucα	GD _{1b}	GD1B	Galβ1-3GalNAcβ1-4Galβ1-4Glc _{2,3} NeuAcα2-8NeuAcα
A hexa	A6	GalNAcα1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,2} Fucα	GT _{1a}	GT1A	NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glc _{2,8} NeuAcα
A hepta	A7	GalNAcα1-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,2} _{1,4} Fucα Fucα	GT _{1b}	GT1B	NeuAcα2-3Galβ1-3GalNAcβ1-4Galβ1-4Glc _{2,3} NeuAcα2-8NeuAcα
Sialylated type 1			Sulphated glycolipids		
3'-sialyllacto- <i>N</i> -tetra	3SLT	NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	Sulphatide	SULF	Gal ₃ HSO ₃
6'-sialyllacto- <i>N</i> -tetra	6SLT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{2,6} NeuAcα	SM3	SM3	Galβ1-4Glc ₃ HSO ₃
3'-sialyl Le ^a penta	3SA5	NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc _{1,4} Fucα	SM2	SM2	GalNAcβ1-4Galβ1-4Glc ₃ HSO ₃
Sulphated type 1			SM1a	SM1A	Galβ1-3GalNAcβ1-4Galβ1-4Glc ₃ HSO ₃
3'-sulphated Le ^a tri	SUA3	Galβ1-3GlcNAc ₃ _{1,4} HSO ₃ Fucα	SB2	SB2	GalNAcβ1-4Galβ1-4Glc ₃ ₃ HSO ₃ HSO ₃
3'-sulphated Le ^a tetra (penta)	SUA4 (SUA5)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc ₃ _{1,4} HSO ₃ Fucα	SB1a	SB1A	Galβ1-3GalNAcβ1-4Galβ1-4Glc ₃ HSO ₃
Neutral type 2			Chondroitin sulphate		
Lactose	LAC	Galβ1-4Glc	Chon O-S	OS	ΔUA1-3GalNAc
Degalactosylated lacto- <i>N</i> -neo-tetra	DLNN	GlcNAcβ1-3Galβ1-4Glc	Chon 4-S	4S	ΔUA1-3GalNAc ₄ HSO ₃
Lacto- <i>N</i> -neo-tetra Le ^a tri (glucosyl)	LNNT LX3	Galβ1-4GlcNAcβ1-3Galβ1-4Glc _{1,3} Fucα	Chon 6-S	6S	ΔUA1-3GalNAc ₆ HSO ₃
Le ^a penta	LX5	Galβ1-4GlcNAcβ1-3Galβ1-4Glc _{1,3} Fucα	Heparin		
Sialylated type 2			Hep IV-A	IVA	ΔUA1-4GlcNAc
3'-sialyllactose	3SL	NeuAcα2-3Galβ1-4Glc	Hep III-A	IIIA	ΔUA1-4GlcNAc ₂ HSO ₃
6'-sialyllactose	6SL	NeuAcα2-6Galβ1-4Glc	Hep II-A	IIA	ΔUA1-4GlcNAc ₆ HSO ₃
Sialyl Le ^a tri (glucosyl)	3SX3	NeuAcα2-3Galβ1-4Glc _{1,3} Fucα	Hep III-S	IIIS	ΔUA1-4GlcN ₂ HSO ₃
3'-sialyl Le ^a penta	3SX5	NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc _{1,3} Fucα	Hep I-S	IS	ΔUA1-4GlcN ₂ ₆ HSO ₃ HSO ₃
6'-sialyl Le ^a penta	6SX5	NeuAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc _{1,3} Fucα	Hep tetra	IS2	ΔUA1-4GlcNAc1-4IdoAα1-4GlcN ₂ ₆ ₂ ₆ HSO ₃ HSO ₃ HSO ₃ HSO ₃
Sulphated type 2			Phosphorylated		
HNK-1	HNK1	GlcAβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc ₃ HSO ₃	Mannose-6- phosphate	MP	Man ₆ H ₂ PO ₃
3'-sulphated Le ^a tri	SUX3	Galβ1-4GlcNAc ₃ _{1,3} HSO ₃ Fucα	Tetramannose phosphate	M4P	Manα1-3Manα1-3Manα1-2Man ₆ H ₂ PO ₃
3'-sulphated Le ^a tetra	SUX4	Galβ1-4GlcNAcβ1-3Gal ₃ _{1,3} HSO ₃ Fucα	Pentamannose phosphate	M5P	Manα1-3Manα1-3Manα1-3Manα1-2Man ₆ H ₂ PO ₃
Gangliosides					
GM ₃	GM3	NeuAcα2-3Galβ1-4Glc			
AsialoGM ₂	GA2	GalNAcβ1-4Galβ1-4Glc			
GM ₂	GM2	GalNAcβ1-4Galβ1-4Glc _{2,3} NeuAcα			
AsialoGM ₁	GA1	Galβ1-3GalNAcβ1-4Galβ1-4Glc			
GM ₁	GM1	Galβ1-3GalNAcβ1-4Galβ1-4Glc _{2,3} NeuAcα			

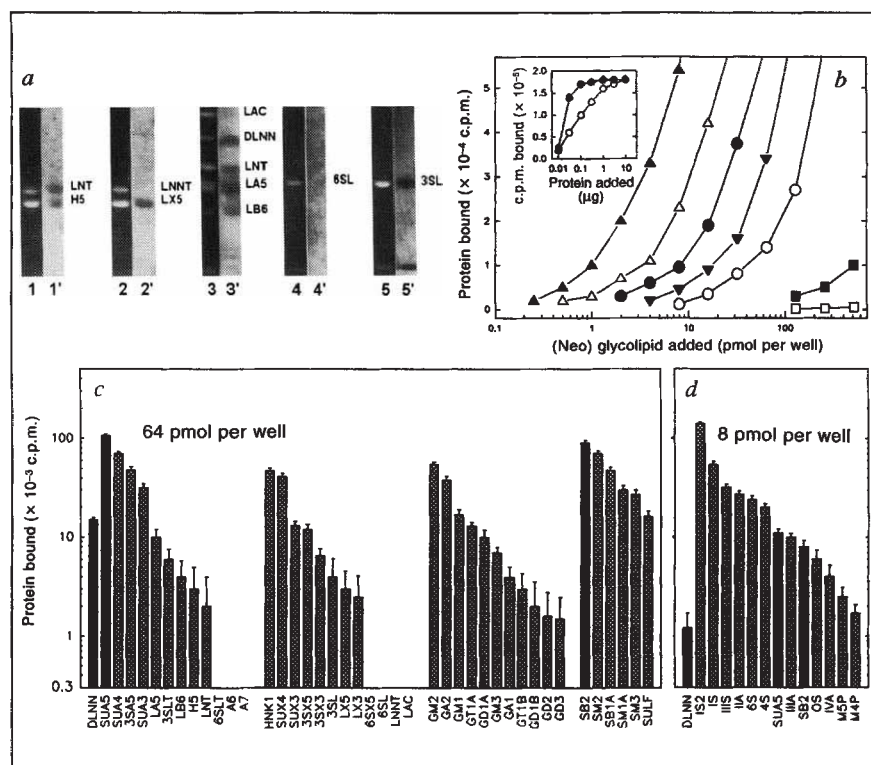
The saccharides shown were investigated as neoglycolipids (oligosaccharides conjugated³⁶ to the aminophospholipid L-1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine) except those under the headings gangliosides and sulphated glycolipids which were natural glycolipids, and the compounds 3SX5 and 6SX5 under sialylated type 2, which were chemically synthesized glycosphingolipids. A proportion of these saccharides (Fig. 2a, b) and mannose-6-phosphate were also investigated as free saccharides. Saccharides under the headings neutral, sialylated and sulphated (types 1 and 2 of the blood group family) are described in refs 18, 37–39, except 3SL and 6SL, which were from Sigma; gangliosides, sulphated glycolipids and phosphorylated saccharides in ref. 40, except the free oligosaccharides of GM2 and GM1 (ref. 41); the tetrasaccharide IS2 was isolated from porcine intestinal heparin (Sigma) after digestion with heparinase I (Sigma) followed by strong anion-exchange HPLC (unpublished); chondroitin sulphate and heparin disaccharides were from Sigma. Abbreviations: Fuc, fucose; Gal, galactose; GalNAc, *N*-acetyl-galactosamine; Glc, glucose; GlcN, glucosamine; GlcNAc, *N*-acetylglucosamine; GlcA, glucuronic acid; IdoA, Iduronic acid; Man, mannose; NeuAc, *N*-acetylneuraminic acid; UA, uronic acid (glucuronic or iduronic, unspecified); ΔUA, 4,5-unsaturated uronic acid.

3Gal α 1-4Gal β 1-4Glc, was bound by NKR-P1, whereas Forsman glycolipid (GalNAc α 1-3-linked to globoside) was not bound. Thus, the affinity of NKR-P1 for *N*-acetylgalactosamine¹⁸ appears to be restricted to the β -anomer (see also ganglio series saccharides below). An affinity for *N*-acetylneuraminic

acid, α 2-3- (but not α 2-6-) linked, was also revealed, as there was binding to 3SL (lane 5') but not to 6SL (lane 4'), nor to lactose (lane 3').

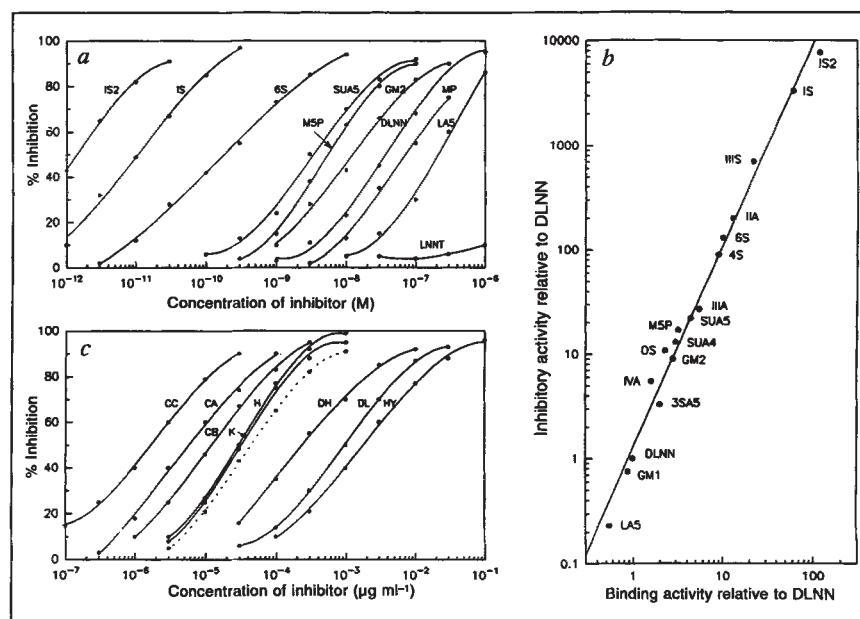
Binding of sNKR-P1 to lipid-linked oligosaccharides immobilized on plastic microwells was saturable (Fig. 1b, inset). The

FIG. 1 Binding of radioiodinated NKR-P1 protein to lipid-linked oligosaccharides. The oligosaccharide sequences and their abbreviations are shown in Table 1. **a**, Neoglycolipids^{36,42}, about 500 pmol of each, were chromatographed³⁶ on silica gel plates in chloroform/methanol/water, 55/45/10 by volume (lanes 3 and 3') or 65/35/8 by volume (other lanes); they were either stained chemically with orcinol (negative images shown in lanes 1 to 5), or overlaid with ¹²⁵I-labelled sNKR-P1 (a recombinant, soluble, dimeric form of NKR-P1 expressed in *E. coli*; originally designated NKR-341), 5×10^5 c.p.m. ml⁻¹ (specific radioactivity 10^7 c.p.m. μ g⁻¹)¹⁸ after treatment of the plates with 0.2% (w/v) polymethacrylate, and binding was detected³⁶ by autoradiography (lanes 1' to 5'). Lanes 1 and 1' contained LNT and H5; lanes 2 and 2' LNNT and LX5; lanes 3 and 3', lactose (LAC), DLNN, LNT, LA5 and LB6; lanes 4 and 4', 6SL; lanes 5 and 5', 3SL; chromatography was upward. **b**, Lipid-linked oligosaccharides (glycolipids or neoglycolipids) were immobilized on plastic microwells in the presence of the carrier lipids cholesterol and egg lecithin^{38,39}, and their interactions with ¹²⁵I-labelled sNKR-P1 protein were determined¹⁸. In the inset, to demonstrate saturability of binding, ¹²⁵I-labelled sNKR-P1 was mixed with unlabelled protein to give 10^6 c.p.m. μ g⁻¹, and applied at increasing levels to wells coated with the lipid-linked oligosaccharides GM2 and DLNN (2 pmol applied per well). In the main panel, the ascending parts of the binding curves are shown for the serial dilutions of selected lipid-linked oligosaccharides applied onto the wells, and overlaid with ¹²⁵I-sNKR-P1, 3×10^5 c.p.m. per well. $\square$, LNNT; $\blacksquare$, LNT; $\circ$, DLNN; $\blacktriangledown$, GA2; $\bullet$, GM2; $\triangle$, SUA5; $\blacktriangle$, IS. In **c** and **d**, the intensities of sNKR-P1 binding



to lipid-linked oligosaccharides are compared by reading off, from the binding curves in **b**, and others not shown, the counts bound at coating levels of 64 pmol per well (**c**) and 8 pmol per well (**d**); results shown are means of duplicates with range indicated by error bars.

FIG. 2 Inhibition of the binding of radioiodinated sNKR-P1 to immobilized DLNN by free oligosaccharides and polysaccharides. **a**, **c**, DLNN neoglycolipid was coated onto microtitre wells (64 pmol applied per well), and the binding of ¹²⁵I-sNKR-P1 (5×10^4 c.p.m. added per well) was measured in the presence of the indicated concentrations of saccharides. Abbreviation for mono- or oligosaccharides are given in Table 1; those for the polysaccharides are: CA and CB, chondroitin sulphates A and B (bovine), respectively; CC, chondroitin sulphate C (shark); H, heparin (from porcine intestinal mucosa); DH and DL, high- and low-*M*, dextran sulphates (average *M*, 500K and 5K, respectively); HY, hyaluronic acid, all from Sigma Chemical Company; K, keratan sulphate (from bovine cornea)²⁰; the dotted curve in **c** is for the heparin tetrasaccharide IS2 expressed for comparison as μ g ml⁻¹. In **b**, the correlation is shown between the activities of the several oligosaccharides examined both in binding and inhibition assays. Binding activities were calculated as ratios of counts bound to the specified immobilized oligosaccharides over those bound to immobilized DLNN (taken from Fig. 1 at 64 or 8 pmol per well). Inhibitory activities of free oligosaccharides relative to that of DLNN were calculated as ratios of the IC₅₀ for DLNN



over those for the specified oligosaccharides; the IC₅₀ values were derived from the inhibition curves shown in **a**, and others not shown.

binding data in Fig. 1*b* and *c* not only corroborated the chromatogram-binding data, but showed in addition that (1) α 1-3-linked fucose confers a binding activity to the lactosyl backbone as in LX3, (2) α 2-3-linked *N*-acetylneuraminic acid potentiates the binding to LNT and to the Le^a and Le^x sequences as in 3SLT, 3SA5 and 3SX5, and (3) α 2-6-linked sialic acid on 6SLT is not bound; moreover binding is abrogated to the Le^x sequence by the presence of α 2-6-linked sialic acid as in 6SX5. Sulphate on galactose (3-*O*-substituted) had an even greater potentiating effect on NKR-P1 binding to the Le^x and Le^a sequences: compare 3SX3 with SUX3 and 3SA5 with SUA5 (Fig. 1*c*). Oligosaccharide chain length influenced intensity of sNKR-P1 binding: the binding to SUX4 was greater than to SUX3, as was the binding to SUA5 greater than to SUA4. A 3-*O*-sulphated glucuronic acid joined by 3-linkage to the outer galactose of LNNT, forming the HNK-1 antigen, also conferred considerable binding activity.

sNKR-P1 binding specificity also encompasses the ganglio series (Fig. 1*c*). As predicted from the monosaccharide-binding study¹⁸, the glycolipid GA2 with a terminal β -*N*-acetyl-D-galactosamine was strongly bound. There was also binding to this sequence in the presence of β 1-3-linked galactose as in GA1. There was a stronger binding when these two backbones were substituted with α 2-3-linked sialic acid: compare GM1 with GA1, and GM2 with GA2. The presence of a second sialic acid, 2-8-linked, resulted in diminished binding: compare GD3 with GM3, GD2 with GM2, GD1B with GM1, and GT1B with GD1A. As with the blood group series, sulphate (3-*O*-substituted) had a greater potentiating effect than 3-linked sialic acid: compare SM3 with GM3, SM2 with GM2, SM1A with GM1, and SB1A with GD1A. Sulphatide (SULF) was also bound. The disulphated glycolipid SB2 with 3-*O*-sulphate both on *N*-acetylglucosamine and galactose was the most strongly bound among the ganglio series glycolipids.

Even stronger NKR-P1 binding was observed among oligosaccharides derived from heparin and chondroitin sulphate (Fig. 1*d*). The non-sulphated disaccharides OS and IVA supported binding, but the analogues containing variously sulphated hexosamines (2-*N*-sulphated glucosamine; 4-*O*-sulphated *N*-acetylglucosamine; 6-*O*-sulphated *N*-acetylglucosamine or *N*-acetylglucosamine) or 2-*O*-sulphated uronic acids (see Table 1) elicited pronounced binding. The 4-*O*- and 6-*O*-sulphated *N*-acetylhexosamines were especially reactive. Among the disaccharides, the trisulphated IS from heparin was the most strongly bound. The strongest binding was to the hexasulphated heparin tetrasaccharide IS2. The 6-phosphorylated tetra- and pentasaccharides, M4P and M5P, also supported binding.

Oligosaccharides bound by sNKR-P1 inhibited the binding of this protein (Fig. 2*a*). When DLNN was used as the immobilized ligand, the *N*-acetylglucosamine-terminating and fucose-terminating oligosaccharides, DLNN and LA5, respectively, were about 10 times more potent inhibitors of binding (IC_{50} 3×10^{-8} M and 2×10^{-7} M, respectively) than the monosaccharides, *N*-acetylglucosamine and fucose investigated earlier¹⁸. The sialylated 3SA5 and the sulphated SUA5 were about 10 times and 100 times more potent than LA5, with IC_{50} values 1.3×10^{-8} M (not shown) and 3×10^{-9} M, respectively. Also, mannose-6-phosphate (IC_{50} of 6×10^{-8} M) was almost 20,000 times more active than the unsubstituted mannose (IC_{50} of 10^{-3} M; ref. 18) and there was a further 10-fold increase in potency (IC_{50} of 5×10^{-9} M) in the presence of an extended mannosyl backbone as in M5P. The highest inhibitory activities were observed with the sulphated oligosaccharides from heparin and chondroitin sulphate (Fig. 2*a, b*); the IC_{50} values for the most potent among these, IS and IS2, were 10^{-11} M and 1.3×10^{-12} M, respectively. Higher concentrations of the oligosaccharides were required for inhibition when the acidic com-

pounds, GM2 glycolipid or IS neoglycolipid were used as coats rather than DLNN neoglycolipid (Fig. 3*a*).

Heparin glycosaminoglycan was only marginally more active as an inhibitor per unit weight than the fully sulphated tetrasaccharide IS2 (IC_{50} of 30 μ g ml⁻¹ and 40 μ g ml⁻¹, respectively; Fig. 2*c*). The keratan sulphate preparation had an activity comparable to that of heparin, indicating that 6-*O*-sulphated galactose^{19,20} is also recognized by NKR-P1. The chondroitin sulphate preparations were superior inhibitors, with IC_{50} values of 2, 5 and 10 μ g ml⁻¹ for chondroitin sulphates C, A and B, respectively. The high- and low-molecular-mass forms of dextran sulphate, and hyaluronic acid, were the least active, with IC_{50} values of 0.2 ng ml⁻¹, 1 ng ml⁻¹ and 1.8 ng ml⁻¹, respectively.

Saccharide-inhibitable sNKR-P1 binding to cells

¹²⁵I-labelled sNKR-P1 bound to the tumour cell lines YAC-1, B16S and 1C21, which are known targets for NK cells²¹; there was negligible binding to the NK-resistant P815 cell line (Fig. 3*b*). The binding was inhibitable with unlabelled sNKR-P1 and with saccharides (Fig. 3*c*). Hierarchy of inhibitory activities of the four oligosaccharides was similar to that observed using immobilized oligosaccharides (compare Fig. 3*a* and *c*). Using YAC-1 cells, the IC_{50} values for the oligosaccharides were comparable with those using the glycosaminoglycan disaccharide IS as coat (Fig. 3*c*); with B16S cells, IC_{50} values were reached with IS and GM2 oligosaccharides, but not with IVA and DLNN; with 1C21 cells, there was inhibition with IS but not with the other oligosaccharides. These results suggest that there may be some differences in the repertoire of acidic oligosaccharide ligands on each of the three target cell lines.

Saccharide-inhibitable killing of target cells

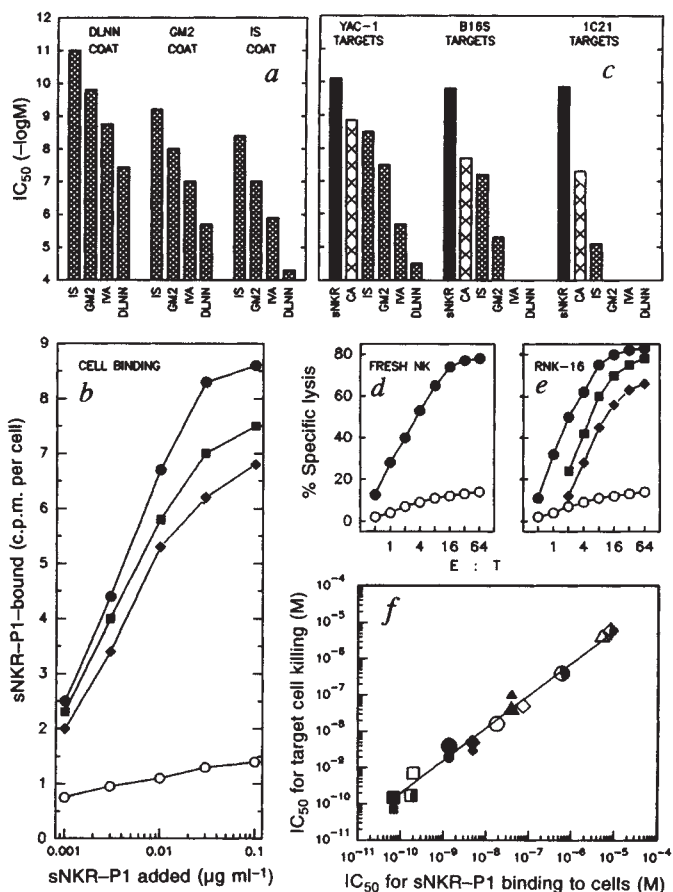
sNKR-P1 protein did not elicit detectable lysis when added to YAC-1, B16S or 1C21 cells at 0.1 to 100 μ g ml⁻¹ (about 2×10^{-9} to 2×10^{-6} M) under conditions of NK lysis assay (not shown). However, lysis of YAC-1 cells both by fresh NK cells and RNK-16 cells, as well as the lysis of B16S and 1C21 cells by RNK-16 cells (Fig. 3*d, e*), was inhibitable by not only sNKR-P1 but also by saccharide ligands for this protein. The inhibition data for the fresh NK cells and RNK-16 cells were similar (shown for YAC-1 target cells in Fig. 3*f*). The IC_{50} values for the inhibition of killing as well as the relative activities of the individual inhibitors differed for the three target cells tested with RNK-16 cells (Fig. 3*f*), and the hierarchy of inhibitory activities was similar to that observed with inhibition of sNKR-P1 binding to these cells. There was a striking correlation between the concentrations of oligosaccharides required for inhibition of sNKR-P1 binding to the target cells and for inhibition of killing of a given target cell by both fresh NK cells and the RNK-16 cell line (Fig. 3*f*). These results clearly implicate NKR-P1 protein (or other proteins with the same specificities) in events leading to the tumour-cell killing process.

Oligosaccharide-mediated activation of NK cells

As expected¹⁷, inositol trisphosphate (InsP₃) and inositol biphosphate (InsP₂) levels increased in RNK-16 cells on exposure to YAC-1 target cells (Fig. 4*a, b*) or when the membrane-associated NKR-P1 molecules were crosslinked with antibody (Fig. 4*g, h*). The responses to YAC-1 cells were suppressed in the presence of sNKR-P1 and the heparin disaccharide IS (Fig. 4*a, b*) at 10^{-8} M and 10^{-7} M, respectively. The elevation of free cytoplasmic calcium on exposure to YAC-1 cells (Fig. 4*c*) was also suppressed in the presence of sNKR-P1, indicating that interactions of the membrane-associated NKR-P1 (or similar proteins) with ligands at the tumour cell surface are intimately involved in the triggering of NK cell activation. In accord with this notion, liposomes containing the disaccharide IS neoglycolipid or GM2 glycolipid, when added to the RNK-16 cells, elicited pronounced increases in InsP₃ and InsP₂ to levels comparable with those observed after crosslinking membrane-

FIG. 3 Comparisons of potencies of oligosaccharides as inhibitors of the binding of radiolabelled sNKR-P1 to different oligosaccharide coats; and comparisons of potencies of these oligosaccharides and those of unlabelled sNKR-P1 and chondroitin sulphate A (CA) as inhibitors of the binding of 125 I-sNKR-P1 to tumour target cells and of the cytotoxicity of various target cells by NK cells. *a*, Microwells were coated^{38,39} with DLNN neoglycolipid or GM2 glycolipid (64 pmol), or IS neoglycolipid (8 pmol) and the inhibition of binding of 125 I-sNKR-P1 determined¹⁸ in the presence of free oligosaccharides IS, GM2, IVA and DLNN. *b*, Binding of 125 I-sNKR-P1 to suspensions of 5×10^5 cells was measured. ●, YAC-1 cells; ■, B16S cells; ◆, 1C21 cells; ○, P815 cells. *c*, Unlabelled sNKR-P1, chondroitin sulphate A (average *M*, 15K), and oligosaccharides IS, GM2, IVA and DLNN were tested as inhibitors of 125 I-sNKR-P1 binding to the target cells YAC-1, B16S or 1C21. *d*, Cytotoxicities of the NK-sensitive YAC-1 and NK-resistant P815 cells by fresh NK cells, and in *e*, the cytotoxicities of YAC-1, P815 and also the NK-sensitive B16S and 1C21 cells by RNK-16 cells were assayed at different effector:target cell (E:T) ratios. Symbols for target cells are the same as in *b*. *f*, The potencies of sNKR-P1, chondroitin sulphate A, and the oligosaccharides IS and GM2 are compared as inhibitors of sNKR-P1 binding to YAC-1, B16S and 1C21 cells (IC_{50} values taken from *c*) and of the killing of YAC-1 cells by fresh NK cells and killing of YAC-1, B16S and 1C21 cells by RNK-16 cells at E:T ratios given in methods (IC_{50} values given are from experiments not shown). Symbols for inhibitors in *f* are: squares, sNKR-P1; circles, chondroitin sulphate A; diamonds, disaccharide IS; triangles, GM2 tetrasaccharide; and upside-down triangle, DLNN trisaccharide; black, white and right-side shaded symbols are used for YAC-1, B16S and 1C21 cells, respectively; for results using fresh NK cells, small closed symbols are used to distinguish from results using RNK-16 cells.

METHODS. Fresh NK cells were isolated⁴³ from spleens of male Fischer F344 rats. The rat NKR-P1⁺ cell line RNK-16 (ref. 44) was grown *in vitro* in complete RPMI with 25 μ M 2-mercaptoethanol (RPMI/ME). The NK-sensitive target cell lines YAC-1, B16S, 1C21 and the NK-resistant cell line P815 (American Type Culture Collection) were cultured in complete RPMI. For radiobinding studies, cells were washed in RPMI with 10 mM HEPES pH 7.4 (RPMI/HEPES), suspended at 5×10^6 cells per ml, and 100 μ l cell suspensions (in triplicate) were incubated with 125 I-sNKR-P1 at 23 °C for 1 h; cells were washed five times in 1 ml RPMI/HEPES, and bound radioactivity measured. For inhibition of cell-binding experiments, 0.5×10^6 cells were mixed with 125 I-sNKR-P1 at concentrations giving half saturation of binding and with inhibitors (total reaction volume 100 μ l). Spontaneous lysis of tumour cells by fresh NK



cells and RNK-16 cell line was measured in triplicate over a range of E:T ratios, 0.5 to 64:1 by a standard 51 Cr release assay⁴⁵. Cytotoxicity inhibition assays using YAC-1, B16S and 1C21 cells were done at E:T ratios of 2:1, 4:1 and 16:1, respectively.

associated NKR-P1 with antibody (compare Fig. 4*d* and *e* with *g* and *h*). These responses elicited by the clustered oligosaccharides were inhibited if the free oligosaccharides were included in the reaction mixtures (*d* and *e*). Dose-dependent elevation of free cytoplasmic calcium was observed in RNK-16 cells on exposure to GM2 liposomes (Fig. 4*f*). At 0.2 nmol per 100 μ l of liposomes, little change in free cytoplasmic calcium was detected; at 2 nmol (equivalent to the level in the phosphoinositide experiments in *d* and *e*), there was a more pronounced increase than at 20 nmol. This effect on triggering of calcium rise was inhibited when GM2 oligosaccharide or sNKR-P1 were included in the reaction mixture (Fig. 4*f*). In an experiment where RNK-16 cells were exposed to chondroitin sulphate A (10^{-7} M), the levels of $InsP_3$ and $InsP_2$ induced (Fig. 4*g*, *h*) were of the same order as those observed with the target cells shown in *a* and *b* (note different scales in *a*, *b* and *g*, *h*); and there was a concomitant increase in cytoplasmic calcium (Fig. 4*i*). Contrasting with the striking effects observed with the clustered ligands on liposomes, when the RNK-16 cells were exposed to the free ligand IS, no changes were detected in the inositol phosphate levels (Fig. 4*g*, *h*) although there was a small (but reproducible) elevation in the overall level of cytoplasmic calcium (Fig. 4*i*).

Oligosaccharide-mediated killing

Cytotoxicity assays were done at effector-to-target (E:T) ratios of 16:1, after exposing the NK-resistant P815 cells to liposomes containing NKR-P1 ligands, GM2 ganglioside, or the IS neoglycolipid; lactose neoglycolipid was used as a negative control

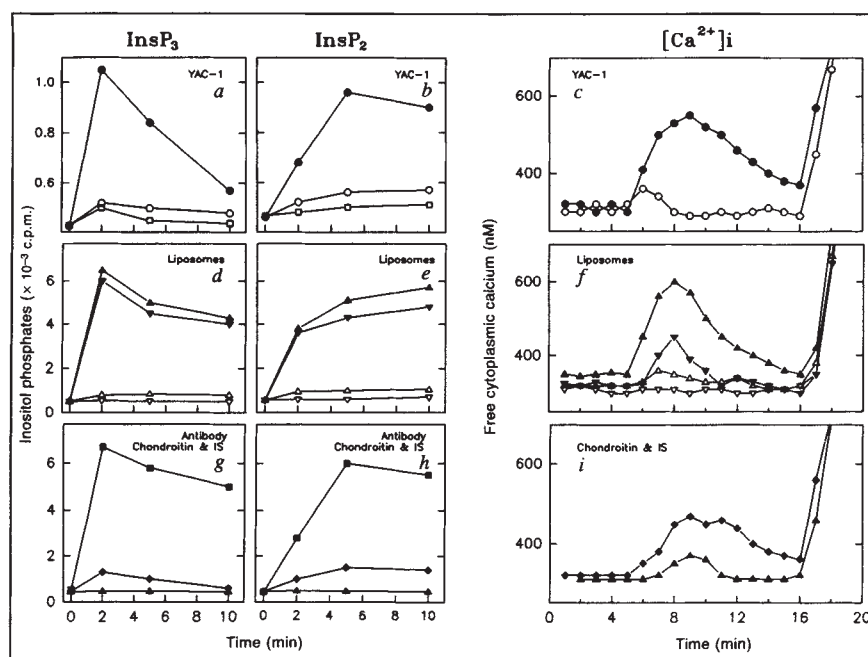
(Fig. 5*a*). Whereas in the absence of liposomes, or in the presence of liposomes containing lactose neoglycolipid, specific lysis of P815 cells was only 10%, this was increased to about 60 and 80% if the P815 cells were preincubated with the GM2 and IS liposomes, respectively. Thus, by pretreating with the NKR-P1 ligands on liposomes, these NK-resistant cells were rendered almost as sensitive to cytotoxicity as the YAC-1 cells at E:T ratio 16:1 (Fig. 3*d*). Preincubation of YAC-1 cells with GM2 and IS liposomes resulted also in increased cytotoxicity of these cells as shown at an E:T ratio of 4:1 (Fig. 5*a*). If after the preincubation step any loosely associated liposomes were washed away from the tumour cells, cytotoxicity of the tumour cells was increased, but to a lesser extent (Fig. 5*a*). If the preincubation step with the liposomes was omitted, only a modest enhancement of the lysis of P815 cells was observed (to about 25% with the GM2 liposomes and 30% with the IS liposomes).

Naturally occurring ligands on target cells

Both sNKR-P1 binding to and cytotoxicity of the YAC-1 cells (Fig. 5*b* and *c*, respectively) were diminished by sialidase and chondroitinase treatments; a mixture of the two enzymes had the greatest effect, with a diminution of sNKR-P1 binding by about 60%, and a diminution of specific cytotoxicity by about 50% of the control values, whereas heparinase I (and heparinase III; data not shown) had little effect.

With the YAC-1 cells, the chondroitinase-susceptible ligands were found to be associated with macromolecules behaving as proteoglycans (Fig. 5*d*): three groups of highly polydisperse components could be discerned migrating as >200K, 150–90K

FIG. 4 Involvement of carbohydrate-protein interactions in activation of NK cells. Levels of InsP_3 (a, d, g), InsP_2 (b, e and h), and free cytoplasmic calcium $[\text{Ca}^{2+}]_i$ (c, f, i) were measured in RNK-16 effector cells after their interaction with YAC-1 cells (a-c), or with liposomes containing oligosaccharide ligands for NKR-P1 (d-f), or with monoclonal anti-NKR-P1 antibody, or chondroitin sulphate A or the free disaccharide IS (g-i). a, b, Amounts of inositol phosphates generated were assayed¹⁷ when $\text{myo}-[^3\text{H}]$ inositol-labelled RNK-16 cells (5×10^6 in 0.25 ml of RPMI/ME) were mixed with an equal number of YAC-1 cells (0.25 ml) in the absence of inhibitors (black circles), and in the presence of IS disaccharide (10^{-7} M) or sNKR-P1 protein (10^{-8} M) (white circles and squares, respectively). d, e, Inositol phosphates were assayed when RNK-16 cells (5×10^6 in 0.45 ml of RPMI/ME) were incubated with 50 μl of liposomes⁴⁶ consisting of 25 nmol each of cholesterol and egg lecithin with incorporated IS neoglycolipid (2.5 nmol, black triangles) or GM2 glycolipid (1 nmol, black upside-down triangles). In parallel experiments (corresponding white symbols) the incubation mixtures also contained the free oligosaccharides IS and GM2, (10^{-7} and 10^{-6} M, respectively). g, h, Inositol phosphates were assayed¹⁷ when $\text{myo}-[^3\text{H}]$ inositol-labelled RNK-16 cells (5×10^6 in 0.5 ml RPMI/ME) were incubated with 0.5 μg of F(ab')_2 fragments¹⁷ of the purified monoclonal antibody 3.2.3 and then crosslinked¹⁶ by addition of 1 $\mu\text{g ml}^{-1}$ F(ab')_2 of goat anti-mouse immunoglobulin (Capell Inc.) (black squares), or chondroitin sulphate A, 10^{-7} M (black diamonds), or IS disaccharide (10^{-7} M, black triangles). c, f, i, Baseline levels of free cytoplasmic calcium were assayed⁴⁷ for 5 min before the RNK-16 cells loaded with Indo-1 AM were exposed to any additives. The reactants in c consisted of 10^6 RNK-16 cells and the same number of YAC-1 cells in 1 ml of RPMI/ME in the absence of inhibitor (black circles), or in the presence of 10^{-8} M sNKR-P1 which was added before the target cells (white circles). f, The reaction mixture consisted of 10^6



RNK-16 cells in 0.9 ml of RPMI/ME, and 100 μl of liposomes (50 nmol each of cholesterol and egg lecithin) and GM2 glycolipid, 0.2 nmol (white triangles), or 2 nmol (black triangles), or 20 nmol (black upside-down triangles) or 2 nmol in the presence of GM2 tetrasaccharide (10^{-6} M) or sNKR-P1 protein (10^{-8} M) (white upside-down triangles). Results with the GM2 tetrasaccharide are shown; those with sNKR-P1 were similar. i, The reaction mixture consisted of 10^6 cells in 1 ml of RPMI/ME and 10^{-7} M chondroitin sulphate A (filled diamonds) or IS disaccharide (filled triangles). At 15 min the incubation mixtures were exposed to 1 μM ionomycin to obtain maximum stimulation of calcium release from intracellular stores.

and <50K. P815 cells contained only trace amounts of these components. In lipid extracts from YAC-1 cells but not P815 cells (Fig. 5e), two major and three minor species were found to express NKR-P1 ligands, and they were identified by their chromatographic mobilities relative to authentic glycolipid standards, and the quasimolecular $[\text{M}-\text{H}]^-$ and fragment ions in their mass spectra (manuscript in preparation). The two most abundant and strongly bound components were identified as GD1A and GM1 gangliosides, each with ceramides containing 36 or 38 carbon atoms; two minor components also bound (arrowed) were identified as GT1B and GD1B gangliosides. The fifth, although relatively strongly bound, corresponded to a trace component in the chromatogram; the molecular ion species recorded in this region of the chromatogram was that for a lactonized form of GD1, and it was tentatively assigned as the GD1A analogue on the basis of its chromatographic mobility relative to GD1B lactone, and the relative mobilities of GD1A and GD1B (Fig. 5e).

Discussion

Our results establish (1) that the membrane-associated NKR-P1 on NK cells is a key effector whose interactions with saccharide ligands on target cells result in killer-cell activation as a lead to target-cell killing; and (2) that gangliosides and glycosaminoglycans, particularly of the chondroitin sulphate type, carry ligands on NK-susceptible tumour cells.

A role for NKR-P1 in the killing cascade could not be demonstrated by others²² using a monoclonal antibody 3.2.3 against NKR-P1 to block killing. A likely explanation is that multiple isotypes of NKR-P1 (refs 11, 15) may operate at the killer-cell surface. These may have similar binding specificities towards

ligands on target cells, but their binding would not necessarily be blocked by antibody 3.2.3 if it is highly specific for one NKR-P1 isotype. Involvement of carbohydrates in NK cell interactions with target cells has long been suspected²³ but there has been little consensus regarding mechanisms for specific oligosaccharides. In the light of our findings, a possible explanation is that different classes of oligosaccharides constitute ligands for NKR-P1, depending on the target cell type.

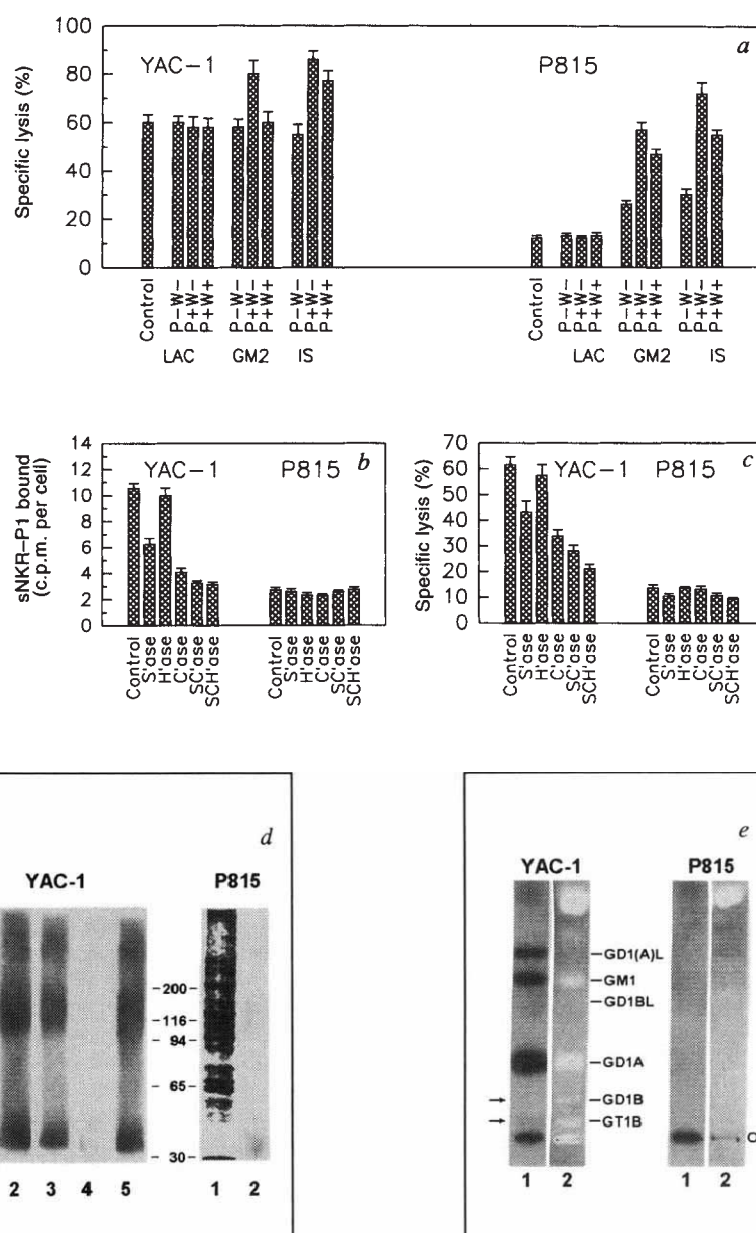
Multispecificity for oligosaccharides, a feature shared with other endogenous lectins (for example selectins, conglutinin and mannan-binding proteins (reviewed in ref. 6)) is unusually pronounced in the case of NKR-P1. The extensive crossreactions observed with NKR-P1 may be a reflection of the high affinities of the CRD of this protein both for calcium and carbohydrates. So far the highest affinities observed are towards the acidic oligosaccharides of chondroitin sulphate, heparin and keratan sulphate types.

It is interesting to note that some of the compounds that have been shown here to express NKR-P1 ligands, for example GM2 and heparin, have been reported previously to inhibit natural killing^{24,26}. Susceptibility of YAC-1 cell variants to NK lysis could be correlated with their expression of GM1, GD1A, GD1B and a GT (ref. 27), and susceptibility of human leukaemia and lymphoma cells was correlated with expression of GM2 (ref. 28). Our results offer an explanation for the intriguing observations^{29,30} that natural killing is inhibitable in the presence of mannose-6-phosphate (and hexose-6-sulphates), a phenomenon later shown not to involve the mannose-6-phosphate receptor^{31,32}.

Several of the NKR-P1 ligands identified in the present study have been detected immunochemically at the surface of NK cells.

FIG. 5 Demonstration that membrane-associated sialyl-glycolipid and glycosaminoglycan type components on tumour cells are biologically relevant ligands for NKR-P1. **a**, Cytolysis of YAC-1 or P815 cells by fresh NK cells were assayed with or without their pre-incubation with liposomes (P+ or P-, respectively) and with or without washing of the cells after the incubations (W+ or W-, respectively). Liposomes containing lactose neoglycolipid (LAC), or GM2 ganglioside (GM2) or the IS neoglycolipid (IS) were used. Control experiments were done in the absence of liposomes. **b**, Binding of 125 I-sNKR-P1 was measured to 5×10^5 YAC-1 cells or P815 cells, which had been treated with sialidase (S'ase) or heparinase I (H'ase) or chondroitinase ABC (C'ase) or a mixture of sialidase and chondroitinase ABC (SC'ase) or a mixture of sialidase, chondroitinase ABC and heparinase I (SCH'ase), or a mixture of the heat-inactivated enzymes (control). **c**, Cytolysis of YAC-1 and P815 cells by fresh NK cells were assayed after treatment of the tumour cells, as in **b**, with the glycosylhydrolases or the inactivated enzymes. **d**, Plasma membranes of YAC-1 and P815 cells were subjected to SDS-polyacrylamide gel electrophoresis and electrotransferred onto nitrocellulose; strips were stained for protein (lanes 1) or overlaid with 125 I-sNKR-P1 to reveal, by autoradiography (at 3 h), components bound (lanes 2); with YAC-1 cell membranes, additional strips (lanes 3, 4 and 5) were tested for binding after treatment with sialidase, chondroitinase ABC or heparinase I, respectively. The positions of M_r markers (K) are indicated. **e**, Lanes 1, binding of 125 I-sNKR-P1 was evaluated to lipids extracted from YAC-1 and P815 membranes resolved by TLC. Replicate strips, lanes 2, were stained with primulin. Circle indicates position of application; GM1, GD1A, GD1B, GD1B lactone (GD1BL) and GT1B indicate the positions of authentic standards. GD1(A)L is a minor glycolipid component tentatively assigned as GD1A lactone. Binding to the relatively low abundance glycolipids GT1B and GD1B was of weak intensity (arrowed) in the 3 h autoradiogram.

METHODS. In the liposome exposure experiments, YAC-1 or P815 cells (10^5 cells in 0.9 ml of complete RPMI) were mixed with 0.1 ml of liposome suspensions prepared as in Fig. 4 legend, and containing 2 nmol of GM2 glycolipid or neoglycolipids of IS or lactose; the mixtures were incubated for 1 h at 37 °C, and the liposome-treated tumour cells were used in the cytotoxicity assays after washing three times with complete RPMI, or without washing. Alternatively, the tumour cells were incubated for 1 h in the absence of liposomes and the liposomes were added to the cell suspensions immediately before the addition of effector cells. As controls, tumour cells were incubated in medium only. Specific lysis of the tumour cells was assayed using fresh NK cells as described in Fig. 3 legend at E:T ratios 4:1 for YAC-1 cells and 16:1 for P815 cells. For glycosylhydrolase treatments, washed cells (10^7) were resuspended in 0.9 ml of 0.01 M phosphate buffer pH 6.5 containing 0.14 M NaCl; enzymes (0.1 U of *Arthrobacter* sialidase, or 2 U of chondroitinase ABC, both from Boehringer Mannheim, or 100 U of heparinase I or 100 U heparinase III (Sigma) or mixtures of enzymes) were added in 0.1 ml of the same buffer, and incubated for 30 min at 37 °C. As controls, the cells were incubated with heat-inactivated enzymes. After these treatments, tumour cells were washed three times in RPMI, and used for binding assays with 125 I-sNKR-P1, or for cytotoxicity assays with fresh NK cells as described in Fig. 3 legend. The results in **a** and **c** are means of triplicates with standard deviations,



and those in **b** are means of duplicates with range indicated by error bars. For identifying components on tumour cells that are bound by 125 I-sNKR-P1, aliquots of the membrane preparations⁴⁸ were boiled in sample buffer⁴⁹, resolved in replicate lanes on 3–7% gradient SDS-polyacrylamide gels (30 µg of membrane protein per lane) and electrotransferred from gels onto nitrocellulose membranes (0.45 µm, BioRad Laboratories, Hercules, CA), cut into parallel strips and one strip was stained for proteins (AuroDye, Amersham, UK). Other strips were blocked in 10 mM Tris-HCl pH 8 containing 150 mM NaCl, 10 mM CaCl₂, 5% BSA and 0.1% Tween 20, and incubated at 20 °C for 1 h with the blocking reagent alone, or sialidase 10 U ml⁻¹ or heparinase I 100 U ml⁻¹ or chondroitinase ABC 1 U ml⁻¹ in 0.01 M phosphate buffer pH 6.5, washed, and binding of 125 I-sNKR-P1 determined under conditions described in legend to Fig. 2a. After lipid extraction⁵⁰ of plasma membranes, extracts were desalted on Sep-Pak Vac C18 cartridges (Millipore) and resolved by thin layer chromatography in chloroform/methanol/0.5% calcium chloride, 55/45/10 (v/v) using 4 µg extract per lane. Lipids were visualized by primulin staining³⁶, and binding of 125 I-sNKR-P1 determined by autoradiography as in legend to Fig. 1a. Components bound were analysed directly on the chromatogram surface by liquid secondary ion mass spectrometry⁴². The GD1B lactone was chemically synthesized (unpublished); a synthetic GD1A lactone was not available.

These include asialo-GM1 (GA1) in the mouse³³ and HNK-1 (ref. 34) and sialyl-Le^x (ref. 35) in the human. These antigens are not among the ligands with highest affinity for NKR-PI (Fig. 1c). Moreover, we have evidence (unpublished observations) that a proportion of the ligands on the NK cell surface are rendered cryptic through saccharide-inhibitable interactions with endogenous membrane-associated components. These features may serve to protect the NK cells from self-cytolysis.

The differing effects of exogenously applied monomeric and clustered oligosaccharide ligands on NK activation are of special relevance to control mechanisms in the cytolytic cascade. The way is now open to identify the ligands for other closely related NK cell proteins, especially Ly-49 of the mouse, and thus shed light on mechanisms by which signals are generated²¹ in Ly-49⁺

NK cells that result in inhibition of the lysis of target cells that express MHC class I antigens, H-2^d or H-2^k.

From the standpoint of drug design it is remarkable that striking negative or positive effects on the cytolytic process can be elicited simply with the free or the lipid-linked forms, respectively, of a heparin disaccharide that lacks¹⁹ anticoagulant activity. Our demonstration that NK-resistant tumour cells can be rendered susceptible by pretreatment with clustered NKR-PI ligands on liposomes offers powerful therapeutic possibilities for *in vivo* purging of selected cells. It can be envisaged that liposomes or other suitable particles loaded with NKR-PI ligands can be guided with antibody mediation to desired target cells for their clearance by NK cells, irrespective of their intrinsic sensitivities to cytolysis by these cells. □

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Dwarf galaxies as the source of X-ray-emitting gas in clusters

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RICH clusters of galaxies contain more hot gas by mass than stars^{1–3}. This gas is not associated with individual galaxies, but instead is smoothly distributed, like the dark matter that is inferred to reside in the clusters. Identifying the origin of this gas will allow us to constrain theories of galaxy formation by placing limits on the efficiency with which gas is converted into stars and on the fraction of the cluster mass that is comprised of normal baryonic matter. Here we suggest that this gas was originally condensed into dwarf galaxies, precursors to the low-surface-brightness dwarf spheroidal galaxies now observed. The condensed gas would have fuelled a burst of star formation, leading to supernova-driven

winds⁴ which could have expelled the gas back into the intracluster-medium. We show that this model can easily account for most of the present X-ray-emitting cluster gas.

We shall therefore consider a scenario in which this gas is ejected from the smallest primaeval galaxies. This contrasts with previous suggestions that the gas comes from the destruction of late-forming giant galaxies⁵ or is primordial. Suppose that some primaeval galaxies have ejected on average a fraction γ of their initial total mass. We require $\gamma < 0.5$; otherwise the virial theorem requires that the ejection unbinds the galaxy. If we can identify which present-epoch galaxies had precursors that behaved this way, then we can use the mass function describing those galaxies to calculate the total gas mass ejected by their precursors. Let us assume that gas is ejected only by the precursors of the dwarf spheroidal (dSph) galaxies. These galaxies have gravitational potentials dominated by dark matter and stellar populations that are not self-gravitating⁶; they are therefore the natural remnants of such an ejection process. They also have extremely small gravitational potential wells so that the gas can escape easily from the galaxies. In clusters, the dSphs have a luminosity function $\phi(L)$ that is well fitted⁷ by a Schechter⁸

function:

$$\phi(L) dL = \phi^* \exp\left(-\frac{L}{L^*}\right) \left(\frac{L}{L^*}\right)^\alpha \frac{dL}{L^*} \quad (1)$$

where ϕ^* is a normalization density, α is the faint-end slope and L^* is a characteristic luminosity which is $\sim 1.4 \times 10^9 h_{50}^{-2} L_\odot$ for dSph galaxies⁹ ($H_0 = 50 h_{50} \text{ km s}^{-1} \text{ Mpc}^{-1}$, and $L_\odot$ is the solar luminosity). The faint-end slope α is a measure of how strongly the number of galaxies increases with decreasing luminosity; a larger $|\alpha|$ corresponds to a steeper slope. Noting that the “fundamental plane” parameter correlations for the dark-matter haloes of late-type and dSph galaxies are two-dimensional over a large luminosity range⁶, we can assume¹⁰ a mass-to-light ratio of the form $(M/L) \propto L^\beta$. The power-law index in this relation, β , measures how rapidly galaxies become dark-matter dominated as their luminosity decreases. Therefore the mass function $\phi(M)$ for dSph galaxies is

$$\begin{aligned} \phi(M) dM = \phi^* \exp\left(-\left(\frac{M}{M^*}\right)^{1/(\beta+1)}\right) \\ \times \left(\frac{M}{M^*}\right)^{(\alpha-\beta)/(\beta+1)} \frac{dM}{(1+\beta)M^*} \end{aligned} \quad (2)$$

where M^* is the mass of an L^* dSph galaxy. The mass of gas ejected from the galaxies is then

$$M_{\text{gas}} = N^* \frac{\gamma}{1-\gamma} \int_{M_-}^{M_+} \frac{M \phi(M)}{\phi^*} dM \quad (3)$$

where the integral is performed over all dSph galaxies, which are assumed to have masses between M_- and M_+ . Here N^* is a characteristic number of contributing galaxies, given by the product of ϕ^* and the cluster volume containing these galaxies. The remaining problems for a given cluster are to determine the values of N^* and M^* and the limits for the integral. Then we can calculate mass M as functions of α , β and γ .

Figure 1 shows the required combinations of α and β if such a model is to explain all the gas in Coma. Coma provides the strongest constraint because the gas there has been measured out to 5 Mpc (ref. 11), which is the farthest out it has been measured in any nearby cluster. Similar results were obtained for other X-ray clusters¹². If the gas mass in Coma is to be explained this way, Fig. 1 shows that a surprisingly steep ($\alpha \leq -1.7$) dSph luminosity function is required. There is evidence for a luminosity function this steep in Virgo¹³, but for rich clusters, published values of α are typically -0.8 to -1.5 . But these values of α are very strongly dominated by parameter coupling between α and the bright-end cut-off luminosity during Schechter-function fitting (even if the dwarfs are treated separately) and do not measure the faint-end slope. A Schechter function fitted to the deepest published data for Coma¹⁴ gives $\alpha = -1.43$ for the dwarfs, but the faint-end slope based on the three faintest points is consistent with $-1.7 > \alpha > -1.9$. The only rich cluster with a published dwarf galaxy luminosity function¹⁵ that goes deep enough to be free of this parameter coupling is Abell 963, which has $\alpha = -1.8$. It is important for the purposes of this work, in which we are interested in the total mass of this population of galaxies, that we know the true faint-end slope. As lower luminosity galaxies are increasingly dark-matter dominated (at blue magnitude) $M_B \approx -8$, Draco and Ursa Minor are at least 99% dark-matter by mass¹⁶, a luminosity function with $\alpha \leq -1.7$ would also imply that most of the dark matter in rich clusters exists as small undetected dark galaxies or their torn-up dark-matter haloes. Furthermore, $\alpha \leq -1.7$ is predicted if dark-matter haloes grow from Press-Schechter¹⁷ condensation given plausible cooling inefficiencies¹⁸ and a perturbation spectrum with index $n = -2$ (for example, cold dark matter) perturbation spectrum¹⁸.

For $-1.9 < \alpha < -1.7$, Fig. 1 implies that $-0.27 > \beta > -0.42$ if $\gamma = 0.33$. The most direct observational constraint on β comes from studies of dark-matter scaling laws⁶; these suggest that for late-type and dSph galaxies, $\beta = -0.22 \pm 0.09$. Assuming a global mass-to-light ratio of 15 for an L^* dSph galaxy and $(B-V) = 0.5$ for dSphs, $-0.27 > \beta > -0.42$ implies a global V-band mass-to-light ratio of 125 to 500 for the faintest dSph galaxies known (Draco and Ursa Minor). These galaxies have V-band mass-to-light ratios of 94 and 83, respectively, within the core-fitting radius¹⁹, but their global mass-to-light ratios depend on the light and dark-matter distributions outside this radius and cannot be measured directly. These distributions have been modelled in detail¹⁶; the best-fitting models allow global V-band mass-to-light ratios of 10^2 to 10^4 . If $-0.27 > \beta > -0.42$, then the total masses of Draco and Ursa Minor are $\sim 10^8 M_\odot$, where $M_\odot$ is the solar mass. This is a factor of 60 less than the maximum mass allowed by modelling of the dark-matter distribution¹⁶ in these galaxies, and a factor of 90 less than the mass limit imposed by their stability against dynamical friction decay into the Galactic halo²⁰.

In summary, the values of α and β that are required for this model to explain the X-ray-emitting gas masses in clusters are found to be consistent with observations; the value of ~ -1.7 is in fact what is predicted if dark-matter haloes grow from hierarchical clustering models. However, the observational constraints on these two parameters are at present uncomfortably few. Therefore we consider other constraints; the strongest are from the cluster mass-to-light ratio and metallicity measurements.

The predicted mass-to-light ratio $(M/L)_{\text{ng}}$ of all cluster

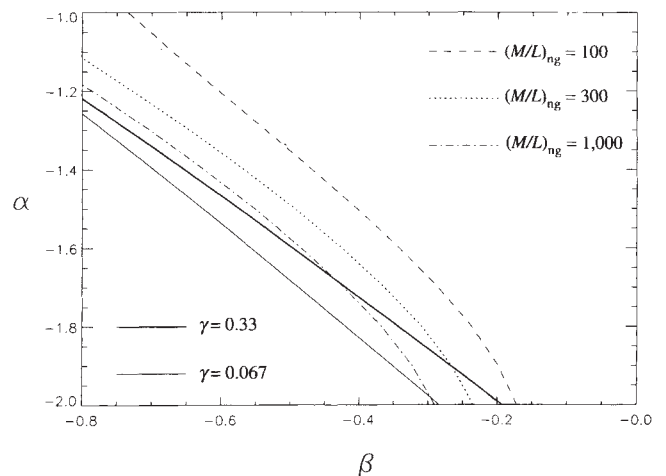


FIG. 1 Combinations of the parameters β (the power-law index in the $(M/L)-L$ relation) and α (the faint-end slope of the dSph luminosity function) that are consistent with the observed gas mass of Coma¹¹ if all of this gas was ejected from dSph galaxies. We assume the dSph galaxies obey a Schechter luminosity function⁸ with $M_B^* = -17.4$ mag (ref. 9) and that the global B-band mass-to-light ratio for an L^* dSph galaxy is 15 (ref. 33). For rich clusters of galaxies, we use a value of N^* equal to that observed in Virgo⁹, corrected for richness using the relative numbers of galaxies brighter than $M_B = -19.6$ mag (refs 9, 34). This assumes that the dwarf-to-giant ratio is the same in rich clusters as it is in Virgo. The fraction of the observed gas mass that is accounted for in these models depends on the Hubble constant as $h_{50}^{1.5}$ where $H_0 = 50 h_{50} \text{ km s}^{-1} \text{ Mpc}^{-1}$. The calculations presented here assume $h_{50} = 1$. The limiting masses for dSphs are taken to be $M_- = 10^4 M_\odot$ and $M_+ = 10^{11} M_\odot$. We display results for the cases $\gamma = 0.33$ (this corresponds to the maximum baryon fraction in Coma¹¹ and thus provides an upper limit on γ) and $\gamma = 0.067$ (this is the minimum gas fraction in giant late-type galaxies³⁵ and therefore a lower limit on γ). Also shown are contours of the mass-to-light ratio $(M/L)_{\text{ng}}$ for the combination of all stars and all dark matter in the cluster that does not reside in the bright ($M_B < -16.7$ mag) galaxies.

material other than the X-ray-emitting gas and the stellar populations of the giant ($M_B < -16.7$ mag) galaxies is also shown in Fig. 1. This includes all the cluster dark matter and also any stars that are in galaxies of sufficiently low surface-brightness that they have not been detected. Observationally, we expect this to be equal to the ratio of the fraction of the cluster dynamical mass in this material (80–85% for $h_{50} \approx 1$)²¹ to the fraction of the total cluster luminosity in this material (15–30%, from measurements of the diffuse background light^{22,23}), multiplied by the total mass-to-light ratio $(M/L)_t$ of the cluster. Assuming^{24,25} that $100 h_{50} < (M/L)_t < 300 h_{50}$ yields $270 < (M/L)_{ng} < 1,700$. This is consistent with the $\gamma = 0.33$ curve in Fig. 1. The total mass (including dark-matter and stars) in the dSphs in Coma would then be $6.1 \times 10^{14} h_{50}^{-1} M_\odot$, half of which is in galaxies with mass below $1 \times 10^5 M_\odot$. This assumes that $M_- = 1 \times 10^4 M_\odot$, that is, the precursors to the dSphs do not form with masses less than $(1 - \gamma)^{-1}$ times this value. If M_- is decreased to $1 \times 10^3 M_\odot$ and α is kept the same, then β increases by 0.02 and half the mass comes from galaxies with mass below $2 \times 10^4 M_\odot$. The results are insensitive to M_+ because of the exponential term in the Schechter function. For $\gamma = 0.33$, the dSphs and their haloes represent about half of the total cluster mass, which is assumed to be $1.1 \times 10^{15} h_{50}^{-1} M_\odot$ (albeit with some uncertainty²¹). This fraction increases for decreasing γ , reaching its maximum permissible value of 85% for $\gamma = 0.22$. For comparison the mass in gas is (ref. 21) $3.1 \times 10^{14} h_{50}^{-2.5} M_\odot$ and in giant galaxies is $2.0 \times 10^{13} h_{50}^{-1} M_\odot$. The agreement between the two sets of curves near $\alpha \approx -1.8$ in Fig. 1 has a further significance. It implies that even if the dSph galaxies have been tidally destroyed, this model can still explain the cluster gas masses. Therefore, even with an observed α much less steep than shown in Fig. 1, most of the gas could originate this way; the galaxies would have since been disrupted and are thus not observed as dSphs. This would also require that the diffuse background light be in loose stars as opposed to faint galaxies. The detection¹³ of a dSph luminosity function with $\alpha \approx -1.7$ in Virgo is evidence that galaxies can form with an extremely steep mass function. Non-detection of such a population in a given X-ray cluster does not necessarily mean that the gas cannot have originated this way, particularly as tidal destruction of dwarfs is very likely in the cores of the richest clusters.

Rich X-ray clusters like Coma have high metallicities, where the metallicity is defined as the abundance of all elements heavier than helium. Metallicities as high as 0.2 solar are typically observed; the origin of these metals has been a source of continuing controversy¹². The strongest observational constraint is the iron abundance. Recent models of galactic winds and supernovae in elliptical galaxies suggest that iron production from these galaxies can explain the cluster iron-mass-to-light ratios^{26,27} (although they appear to have difficulties in explaining the metallicities of several elliptical galaxies^{28–30} unless significant dilution has occurred). However, these models generally require that the gas ejected by the elliptical galaxies is more metal-rich than the intracluster medium (ICM) by a factor >10 . Therefore they fail to explain the origin of at least 90% of the total mass of the ICM. This has led to speculation^{1,26,27} that galaxy formation is very inefficient, and that $<10\%$ of the baryons are incorporated into galaxies. An alternative explanation is that $>90\%$ of the gas condensed into low-mass galaxies, fuelled a burst of star formation, and was expelled by the resulting supernova-driven winds. This gas need not be significantly enriched, as these galaxies have sufficiently small potential wells that the winds from the first burst of star formation are sufficient to expel all the gas in the galaxy⁴. There is independent evidence for this in that lower-mass ellipticals have lower metallicities³¹. Furthermore, if the gas ejection is sufficiently energetic and does indeed occur on a small timescale, it could provide the heat source hypothesized³² to explain the observed trend of the baryon fraction increasing with radius. This is in fact what the present model suggests, although we note that it may be difficult to distinguish

between these two explanations. So, if the dSph galaxies account for more than 90% of the ICM mass, then the models of iron production in elliptical galaxies explain not only the cluster iron masses, but also the actual metallicities. $\square$

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A simple chemical method of opening and filling carbon nanotubes

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SINCE carbon nanotubes¹ were first synthesized in macroscopic quantities², it has become possible to explore their physical and chemical characteristics. There has been much speculation³ about the properties of materials encapsulated within the tubes, but experimental studies of this issue require a reliable means of opening and filling the tubes. Various approaches have been developed for opening up^{4–6} the tube ends and encapsulating material^{4,6,7}, but these work only for a limited range of materials or in low yield. Here we describe a general method that allows carbon nanotubes to be opened at the end and filled with a variety of metal oxides using wet chemical techniques. We anticipate that this method will lead to extensive study of the chemistry and physics of filled nanotubes, which might find applications in catalysis, separation and storage technology and in the development of materials with new magnetic and electrical properties.

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There have previously been attempts to insert materials into carbon nanotubes by using composite metal-carbon electrodes during arc vaporization⁴⁻⁹. This high-temperature method favours the production of metal carbides, and can only be applied to a limited number of materials. In addition, the metal carbides or metal particles are found to be encapsulated by carbon shells, and are thereby isolated from their environment. It has also been reported that if the nanotube is heated in air with lead, the tip of the tube can be opened, followed by the filling of the tube with the molten lead¹⁰. About 1% of the tubes were filled in this manner and the presence of internal caps frequently limit the amount of tube filling. Recently, there have been two reports of the selective oxidation of nanotubes by carbon dioxide¹¹ or oxygen¹² in which ends of the nanotubes could be removed at temperatures $>600^{\circ}\text{C}$ leaving a small proportion (2–10%) of opened tubes. Oxidation in this manner also removes the outer layers of hexagonal carbon which form the body of the tubes. In consequence the tubes become thinner and this can lead to the formation of single-layered tubes¹¹. The filling of open tubes prepared by these gas-phase oxidations has proved difficult, probably because of the presence of amorphous carbon blocking the tubes¹².

Samples of carbon nanotubes were prepared by arc vaporization as described earlier¹¹: this method provides 15 g h^{-1} of sublimed cathodic carbon which contains the nanotubes ($\sim 25\%$) together with other nanoparticles. Samples (2 g) of this carbon were suspended in 45 g concentrated nitric acid (68% AnalaR grade, BDH) and refluxed for 4.5 h in an oil bath maintained at 140°C . A weight loss of less than 2% was recorded. After washing with distilled water and drying at 100°C overnight, the black insoluble product was sonicated in chloroform and dried *in vacuo*. The nanotubes were examined by high-resolution transmission electron microscopy, using an accelerating voltage of 100 kV to avoid beam damage of the carbon material; the microscope was operated at optimum defocus. The micrographs showed that the ends of $\sim 80\%$ of the tubes were opened. About 90% of the tubes were found to be opened in a sample treated with nitric acid for 24 h, although some amorphous carbon was also present. These values were estimated by examining 60 tubes with ends protruding from the sample aggregates. Typical high-resolution electron micrographs of nitric acid treated nanotubes are shown in Fig. 1. Close examination of nanotubes reveals that they have been attacked specifically at those points where the curvature of the tube implies the presence of non-six-membered (probably five-membered) carbon rings (for example, X and Y in Fig. 1a). It is interesting to note that the curved areas of all the internal caps (partitions) are also attacked, giving unobstructed tubes (Fig. 1b, c). The opened tubes appear to be empty. We have also examined the nanoparticles that accompany nanotubes¹³ and found that these are also selectively attacked at the regions of greatest curvature. Because of the

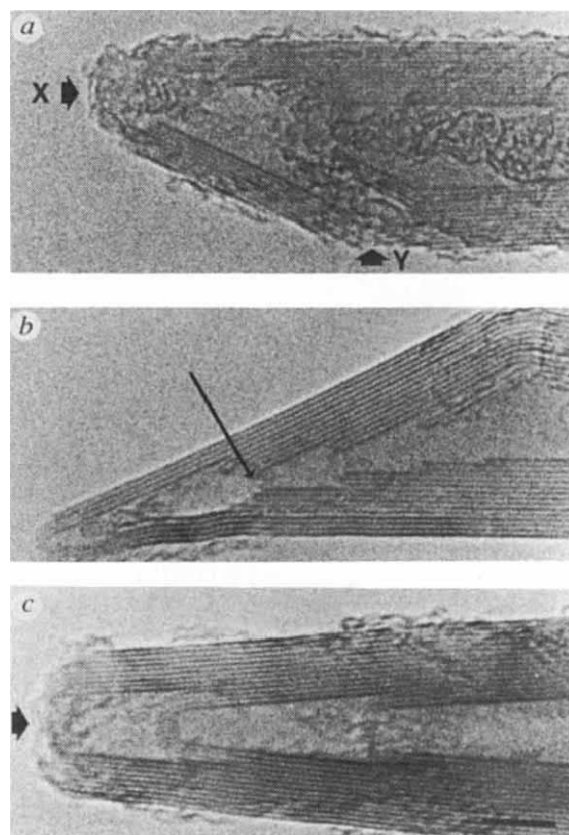
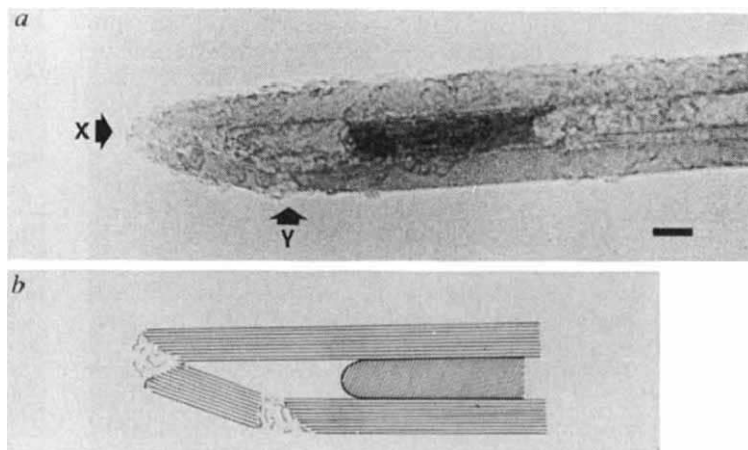


FIG. 1 Typical high-resolution electron micrographs of nitric acid treated nanotubes. *a*, Attack has occurred at points X and Y where non-six-membered carbon rings are present. *b* and *c*, Arrows show sites of the destruction of multiple internal caps by nitric acid treatment. Scale bar, 50 Å.

aggregation of the long nanotubes in the sample, it is difficult to examine both ends of a single tubule. However, the high yield of opened tubes implies that a considerable proportion are uncapped at both ends. Samples of opened tubes are available from the Furnace Chemical Company Ltd, Oxford.

The preparation of filled nanotubes was carried out by suspending 0.4 g capped nanotubes in 20 g of nitric acid solution containing 1 g of hydrated nickel nitrate ($\sim 5\%$ w/w) and refluxing for 4.5 h in an oil bath at 140°C . The suspension was allowed to settle and the supernatant solution was decanted. The result-

FIG. 2 *a*, High-resolution electron micrograph of a nickel-material-filled nanotube also opened at points X and Y; the observed fringes of $2.40 \pm 0.05\text{ Å}$ correspond to (111) NiO crystal planes. Scale bar, 50 Å. *b*, A tracing of the layer planes is provided for clarity.



ing insoluble black product was dried overnight in an oven at 100 °C. The sample was then heated in a stream of helium at a rate of 10 °C min⁻¹ from room temperature to 100 °C and kept at this temperature for 1 h before ramping at 4 °C min⁻¹ to 450 °C. The sample was then annealed for 5 h at 450 °C. Electron micrographs showed that many (~80%) of the nanotubes had been opened by nitric acid treatment, and that of these ~60–70% contained nickel material (Fig. 2). Some nickel-containing material was also observed on the exterior of the nanoparticles and nanotubes. No intercalation of nickel material between carbon layers of the nanotubes was observed. Close examination of the nickel material inside the tubes showed lattice fringes with an observed fringe separation of 2.40 ± 0.05 Å, consistent with the interlayer separation (2.41 Å) of the (111) crystal plane of nickel oxide (NiO). X-ray powder diffraction (not shown) also indicates the presence of nickel oxide. It is interesting to note that the (111) NiO plane is always aligned at ~55–65° to the graphite layers (0002) of the tube. Most of the NiO crystallites have diameters almost equal to the internal cross-section of the tubes (3–6 nm) and have lengths of 10–30 nm. These crystallites can also be observed considerably distant from the opened ends. It can be envisaged that nickel nitrate solution was sucked into the tubes as they were opened and crystalline NiO was formed during calcination. The form of growth of NiO crystallites may be influenced by the surface structure of the inner tube and the elongated shape of the NiO crystallites may reflect surface-wetting properties.

Nanotubes filled with NiO can also be prepared by immersing empty and dried opened tubes in nickel nitrate solution followed by evaporation and calcination as before. However, the percentage of filled tubes is less (~20–30%) than the *in situ* one-step method.

Treatment of closed nanotubes with a nitric acid solution of uranyl nitrate (0.5% w/w) for 4.5 h gave open-ended nanotubes containing uranium. It is estimated that 70% of the tubes contain uranium oxide material. Figure 3 shows crystallites in the inner tube with lattice fringes of 3.15 ± 0.05 Å; these are randomly oriented with respect to the graphite layers. The layer separation corresponds to that reported for (111) UO_{2+x} crystallographic planes. Other interlayer separations determined from the transmission electron microscopy and X-ray powder diffraction spectra are also consistent with the fluorite structure of UO_{2+x} ; the precise value of x is not known. The UO_{2+x} crystallites are ellipsoidal with lengths of 3–8 nm. The observed contact angles, usually >100°, suggest that the uranium oxide crystallites wet the carbon surface poorly. We have found that most of the uranium oxide particles can be dissolved from the inner tubes by treatment with concentrated aqueous nitric acid at room temperature for a few minutes.

Samples of closed nanotubes were also heated in a nitric acid solution containing cobalt nitrate or iron nitrate. Following the standard heat treatment the resulting black residues were shown by electron microscopy and X-ray diffraction to contain oxides of cobalt or iron material, respectively.

The samples containing the oxides of Ni, Co or Fe were

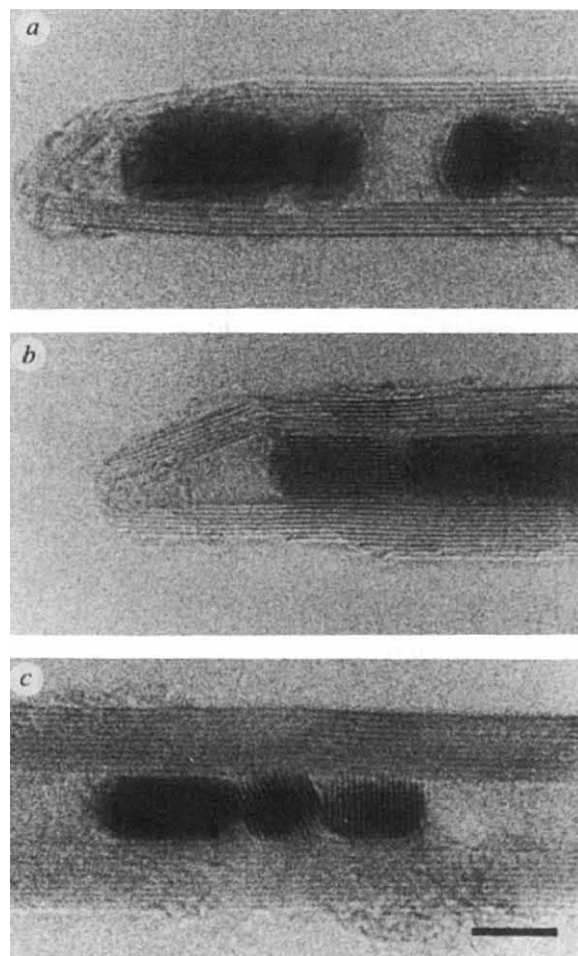


FIG. 3 a–c, High-resolution electron micrographs of nanotubes filled with uranium oxide. The observed fringes of 3.15 ± 0.05 Å correspond to the (111) UO_{2+x} crystal planes (refer to 5-550 JCPDS-ICDD 1990). Scale bar, 50 Å.

treated under hydrogen at 400 °C for 2 h. Close examination of the reduced materials showed a change of morphology of the resulting crystallites and the loss of the lattice fringes associated with the original oxide (NiO). Figure 4 shows the appearance of a crystal formed by reduction of an NiO crystallite. The appearance of facets on the end of the nickel particle is typical for a metallic crystal, and we speculate that the oxide has been reduced to the native metal. Further studies are needed to elucidate the nature and properties of these crystallites. Transmission electron microscopy shows that crystals, for example UO_{2-x} , are deposited along the entire length of the tubes (~1–2,000 Å).

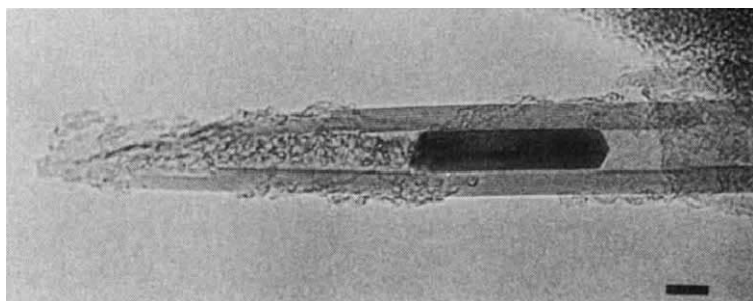


FIG. 4 High-resolution electron micrograph of a nanotube filled with nickel material by hydrogen reduction of a NiO-filled precursor: the faceting of the particles is typical of a single crystal of nickel metal. Scale bar, 50 Å.

This implies that the solution completely fills the tubes. Our method opens the way to filling the internal volume of nanotubes with a wide variety of materials using solutions of their precursors. □

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Role of the thermohaline circulation in the abrupt warming after Heinrich events

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EVIDENCE of rapid climate oscillations during the last glacial period has been identified in climate records from Greenland ice cores^{1,2} and ocean sediments in the North Atlantic^{3,4}. These records show that periods of gradual cooling are terminated by abrupt warming events⁵, with the coldest periods coinciding with the deposition of ice-rafted debris (so-called Heinrich events) throughout the North Atlantic. Heinrich events are thought to be a signature of massive iceberg discharges owing to collapse of the Laurentide ice sheet; Bond *et al.*⁵ have proposed that the decrease in meltwater flux following collapse and retreat of the ice sheet enhanced the ocean's thermohaline circulation⁶, thereby increasing advection of heat from the tropics and giving rise to abrupt climate warming. Here we test this idea using a simple ocean model coupled to a model of a periodically surging ice sheet. We find that massive discharges of icebergs first stop the thermohaline circulation because of the consequent freshwater influx, cooling the North Atlantic region. This is followed by a rapid restart of the circulation, leading to abrupt warming. Thus our model can reproduce the qualitative features of the climate oscillations seen in the ice-core and ocean records.

The so-called 'Dansgaard-Oeschger events' were first seen as abrupt shifts in the oxygen isotope ratio of three Greenland ice cores, from Camp Century, Dye 3 and Renland. Two new cores, GRIP^{1,7} and GISP2⁸, recently confirmed these rapid excursions in $\delta^{18}\text{O}$, which reflect changes in the temperature of formation of local precipitation^{1,9}. Furthermore, marine sediments in many cores over the North Atlantic exhibit layers of detritic materials³. These materials came largely from the margins of the Labrador Basin¹⁰; transport to lower latitudes is explained by enhanced discharge of icebergs and subsequent melting. During these deposition events, the sea surface temperature was considerably cooler^{4,5}. Therefore, both ice-core and marine-sediment data strongly suggest rapid climate oscillations during glacial periods. The periodicities of the 'Dansgaard-Oeschger events' vary from 1 to 10 kyr, and the longer-term cycles (7–10 kyr) culminate at the same time as Heinrich events. Figure 3 of ref. 5 shows that iceberg discharge indeed occurs when temperatures over Greenland, as well as sea surface temperatures, are around their minima. These features are observed throughout the glacial period and even during the deglaciation. Some continental records¹¹ correlate well with these longer-term oscillations. However, the most puzzling feature of these climate proxies is the abrupt warming just after periods of slow cooling. Rapid warming is particularly well defined in the ice-core record: in some cases temperatures rise by several degrees within decades¹.

Orbital variations of insolation are obviously too slow and furthermore too limited in amplitude during the period¹² 60–20 kyr before present (BP) to account for such decade-to-century scale variability. Furthermore, massive iceberg discharge and climate variations seem to be linked in the palaeodata. Recently, model simulations have shown that massive iceberg discharges from the Laurentide ice sheet could not result directly from cooler temperatures over the Northern Hemisphere¹³. On the

FIG. 1 The ocean model is composed of three boxes: 1, high-latitude waters; 2, low-latitude surface; and 3, intermediate plus deep ocean. The fourth reservoir is the ice sheet of height H . The diffusive mixings k_{12} , k_{13} , k_{23} are constant, and the thermohaline circulation m is assumed to be proportional to the density difference between the two surface boxes, using a linear equation of state for sea water $m = \mu[\alpha(T_2 - T_1) - \beta(S_2 - S_1)]$, where T_i and S_i are the temperature and salinity of box i . In the atmosphere, the heat transport is $L = C_L(T_2 - T_1)$ and the water vapour is $V = C_V(T_2 - T_1)$. The snow accumulation is $A = C_A(T_2 - T_1)$, the melting is $M = C_M(T_1 - T_f)$ if $T_1 > T_f$. Where T_f is the melting temperature of ice (here 0°C), $M = 0$ otherwise. Iceberg calving is $I = 0$ when the ice sheet is growing and $I = -V/\tau$ during iceberg discharges. The net radiation fluxes are $Q_1 = Q_1^0 - q_1 T_1$ and $Q_2 = Q_2^0 - q_2 T_2$. The temperature profile in the ice sheet is parametrized by $T(z) = T(H) + \theta^2(1 - z/H)/(\theta + (\gamma - \theta)z/H)$, where $\theta = T(0) - T(H)$, $T(H) = T_1 + C_T - \Gamma H$, and γ is the temperature gradient at the base of the ice sheet imposed by the geothermal flux and, during surges, friction. (See Table 1 for definitions of symbols.)

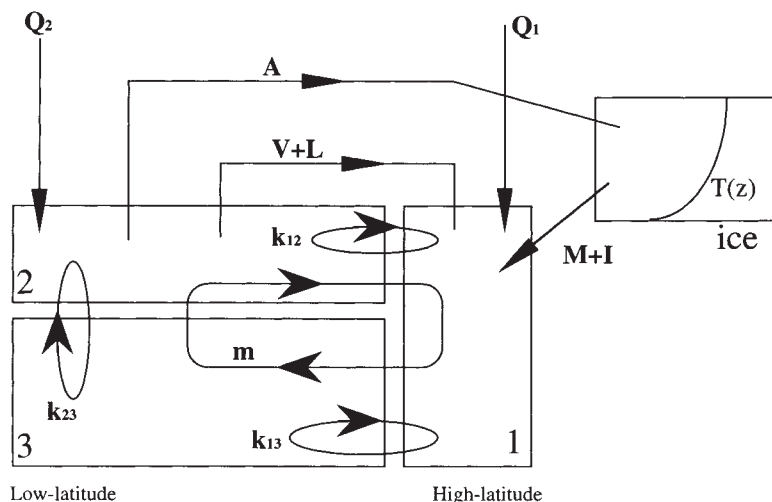


TABLE 1 Parameter values

Parameter	Value	Unit
V_1	20	10^6 km^3
V_2	20	10^6 km^3
V_3	200	10^6 km^3
k_{12}	6	Sv
k_{13}	10	Sv
k_{23}	10	Sv
μ	10,000	$\text{Sv m}^3 \text{ kg}^{-1}$
Q_1^0	-4.8	10^{15} W
Q_2^0	6	10^{15} W
q_1	-0.04	10^{15} W K^{-1}
q_2	-0.04	10^{15} W K^{-1}
F_G	0.05	W m^{-2}
Γ	9	K km^{-1}
C_T	6	K
τ	1,000	yr
C_A	0.0025	Sv K^{-1}
C_M	0.002	Sv K^{-1}
C_L	0.095	10^{15} W K^{-1}
C_V	0.037	Sv K^{-1}

Symbols used: V_1, V_2, V_3 are the volumes of the oceanic boxes; k_{12}, k_{13}, k_{23} the diffusive fluxes; μ the thermohaline circulation coefficient; Q_1^0, Q_2^0, q_1, q_2 the radiative coefficients; F_G the geothermal flux; Γ the atmospheric lapse rate; C_T the continental temperature effect; τ the surge time constant; C_A the accumulation coefficient; C_M the melting coefficient; C_L the meridional heat flux coefficient; C_V the meridional water vapour flux coefficient.

other hand, it has been shown that ice sheets (or at least ice-sheet models) can undergo internal oscillations^{14,15}, and it has been suggested that these oscillations could induce the climate changes associated with Heinrich events^{15,16}.

Such rapid climate warmings are nevertheless rather difficult to explain. The ocean probably plays a considerable part. Simple ocean box-models¹⁷ up to coupled ocean-atmosphere general circulation models¹⁸ show that thermohaline circulation in the North Atlantic region may exhibit two stable regime of flow. An 'off-mode' with a greatly reduced deep-water production in polar regions; and an 'on-mode' with an active deep-water formation and an efficient northward transport of heat and salt: high-latitude temperatures are then higher. A 'turn-on' or a 'turn-off' of the circulation is possible by changing the salinity budget at high latitudes, induced by evaporation, precipitation or runoff. The concept of a 'salt-oscillator' has already been proposed to explain the climate variations found in glacial time^{6,19}: abrupt cooling or warming is possible in only a few decades by changing slightly the freshwater budget.

Following these ideas, two tentative explanations have been proposed to explain the abrupt warming after Heinrich events, both involving an increase in the salinity of the North Atlantic, and thus a strengthening of the thermohaline circulation. Bond *et al.*⁵ suggest that, after the collapse of the ice sheet and the subsequent retreat of the ice margin, the flux of icebergs to the ocean is much reduced and the salinity is then allowed to increase. On the other hand, the fact that the high elevation of the Laurentide ice sheet is responsible for increased northerly winds over the western part of the North Atlantic, which considerably cools the surface waters during glacial time²⁰, led MacAyeal to suggest¹⁶ that its reduced elevation after collapse could induce a warming of surface waters, and consequently enhance the evaporation and the salinity.

Proxies for glacial palaeoceanography²¹ indicate that, during glacial periods, deep-water formation occurred mainly in the North Atlantic around 50° to 60° N. A large proportion of the icebergs associated with Heinrich events will melt in the same general area¹⁰ and will therefore have a considerable influence on the thermohaline engine. Here we explore the idea of Bond *et al.*⁵ using a numerical model coupling an oscillating ice sheet

with a bimodal ocean-atmosphere system. A schematic representation is shown in Fig. 1.

The ice-sheet is a zero-dimensional simplification of MacAyeal's model¹⁵, using a standard Galerkin method. The ice-sheet model oscillates between a growth phase and a surging phase, initiated by basal ice melting and subsequently stopped by basal ice freezing. During ice-sheet growth, we use the full advective-diffusive equation from MacAyeal's model, whereas during surges we consider only the downward advection of ice. The mass balance of the ice sheet is determined by the difference between accumulation and melting plus ablation. The ocean model is derived from Birchfield's three-box model¹⁷. It contains a low-latitude surface, a deep plus intermediate and a high-latitude box as shown in Fig. 1. Equations for temperature and salinity in each box are obtained by taking the balance of sources and sinks. Unlike Birchfield, we use an upstream advection scheme, though this is not crucial for the final result. Using the parameter values listed in Table 1, the model is integrated from an arbitrary initial condition for 50 kyr in order to eliminate transient behaviour, then for another 50 kyr to obtain the oscillations shown in Fig. 2. Vertical density stratification between boxes 2 and 3 always remains stable.

The role of the ocean is most easily seen in Fig. 3. Starting from a growing ice sheet and an active thermohaline circulation, ice-sheet melting brings only small quantities of fresh water to the high-latitude ocean. When the ice sheet collapses due to basal melting, a huge additional freshwater flux from iceberg calving and subsequent melting damps the circulation. This causes high-

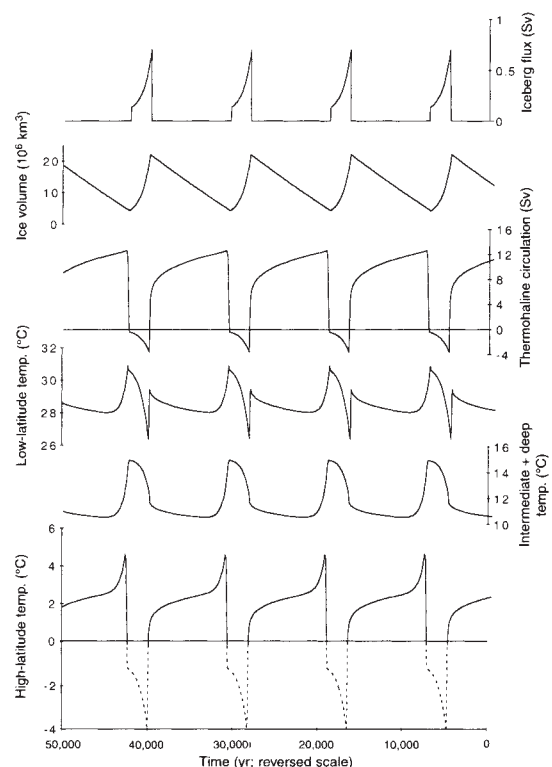


FIG. 2 Time evolution of the iceberg flux (in sverdrups, $10^6 \text{ m}^3 \text{ s}^{-1}$), the ice-sheet volume, the thermohaline circulation, and the temperatures of each box. Massive iceberg discharges stop the thermohaline circulation thus warming the low-latitude boxes while cooling the high-latitude box. The dashed line indicates sea-ice formation, which is equivalent to taking the high-latitude temperature curve as the heat content of the northern box. Just after the 'Heinrich event', the heat stored at low latitudes is brought to higher latitudes, resulting in an abrupt warming (over ~ 100 yr). Afterwards, the system slowly relaxes to what would be the equilibrium in the absence of the ice sheet.

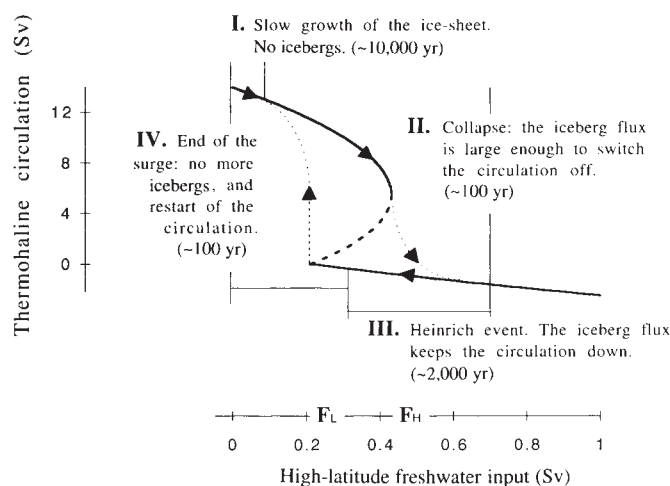


FIG. 3 The equilibrium value of the thermohaline circulation for the three-box ocean-atmosphere model alone (decoupled from the ice-sheet dynamics), plotted here as a function of the non-atmospheric freshwater flux (ice sheet and iceberg melting) to the high-latitude box. The successive phases of one oscillation are indicated. A transition from the 'on-mode' to the 'off-mode' occurs when the iceberg flux exceeds the high-threshold value F_H , and the opposite transition occurs when no more icebergs are produced, the freshwater flux being then below the low-threshold value F_L .

latitude cooling and low-latitude warming (Fig. 2) because of reduced meridional heat exchange: this temporary heat storage in low latitudes is essential for the subsequent warming at high latitudes. When the ice-sheet basal freezing interrupts the iceberg flux, the freshwater input comes back to its initial value and the thermohaline circulation restarts abruptly, bringing to higher latitudes the heat previously stored during the 'off' phase.

The abrupt climate changes present in our model correspond to the transient behaviour of a bimodal atmosphere-ocean system, influenced by periodic massive iceberg discharges. The direct melting of the ice sheet (M in Fig. 1) has here no essential role, in contrast to other models of glacial oscillations^{22,23}. Note also that no attempt whatsoever has been made to fit closely the palaeodata. In particular, the periodicity of the oscillations depends, as in MacAyeal¹⁵, on the parameter values chosen for the geothermal gradient, ice accumulation and ablation, and it varies between 5 and 30 kyr. The value of 11,750 yr found here has no particular significance. Likewise, an iceberg flux of a few tenths of a sverdrup ($1 \text{ Sv} = 10^6 \text{ m}^3 \text{ s}^{-1}$) is compatible with the surface-water $\delta^{18}\text{O}$ excursions of 1–2‰ found during 'Heinrich events'⁵, representing a 20 to 40 times dilution of ice with a $\delta^{18}\text{O}$ of –35‰: for a 100-m-deep surface layer covering $6 \times 10^6 \text{ km}^2$ of the North Atlantic and mixed over a 10-year period, an excess 2–5 m of fresh water corresponds indeed to a 0.5–1 Sv flux.

Thus, perhaps the simplest possible model coupling the ocean-atmosphere system with an ice sheet undergoes oscillations that have some common features with palaeoclimate records. In particular, we observe an abrupt warming in high-latitude regions just after the massive iceberg discharges from the ice sheet. Although this model is probably too simplistic to predict any numerical value, it nevertheless demonstrates the effect that the interactions between ice-sheet dynamics and the deep ocean circulation have on the variability of climate. □

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Melt topology in partially molten mantle peridotite during ductile deformation

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THE process by which basaltic melt is generated and extracted beneath mid-ocean ridges is poorly understood. Knowledge of the distribution of melt within the parent mantle peridotite during the early stages of melting is important for interpretation of geophysical experiments and for construction of models of magma coalescence and extraction^{1–4}. Static experiments on mantle rocks and selected analogue materials have shown that, for small melt fractions, melt is concentrated along three-grain intersections, forming an interconnected web of tubes^{5–9}. Low-pressure deformation experiments on olivine + melt specimens have yielded the same conclusion^{10,11}. But in similar experiments on salt-brine mixtures during ductile deformation, the fluid emerges from the triple junctions where it resides under static conditions and spreads onto grain boundaries^{12–16}. Here we report the results of low-stress deformation experiments on partially molten peridotite at mantle temperatures and pressures, which show that such dynamic melting produces microstructures analogous to those of the salt-brine experiments. The very low viscosity of these specimens suggests that in the Earth, dynamic wetting could lead to melt separation at very low melt fractions, and to melt focusing at ridges.

Although many static partial-melting experiments have been performed on mantle compositions under mantle conditions^{5–9}, previous dynamic experiments have been conducted at ambient or low pressures on bulk chemistries significantly different from the primary peridotite of the Earth's mantle^{10,11,17}, or at stresses ~100 times those expected in the mantle¹⁸. At low pressures, the mineralogy of peridotite is different from that at the depths of melting in the Earth (the pyroxenes and aluminous phases change with pressure), and under high effective stresses melt-induced microcracking occurs. There is therefore a need for dynamic experiments more closely approaching the physical and chemical conditions in the mantle. Taking advantage of our molten-salt cell^{19,20} that allows low-stress deformation to be

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performed under high pressures, we have started such a programme of work^{21,22}.

Our starting materials are a series of synthetic peridotites ranging from pyrolite (52% olivine, 31% orthopyroxene, 14% clinopyroxene, 3% spinel) to harzburgite (81% olivine, 12% orthopyroxene, 4.6% clinopyroxene, 2.4% spinel), all constructed by separation and recombination of crystals from a single lherzolite xenolith from Damaping, eastern China. The resulting mixtures were ground, sized, heated to 373 K in flowing argon, evacuated and isostatically hot pressed at $P=0.1$ GPa, $T=1,475$ K for 1 h in Mo-lined steel containers to produce rocks of grain size 20–50 μm . Here we emphasize early melting of pyrolite, although the more refractory lithologies display similar microstructures at similar melt fractions^{21,22}.

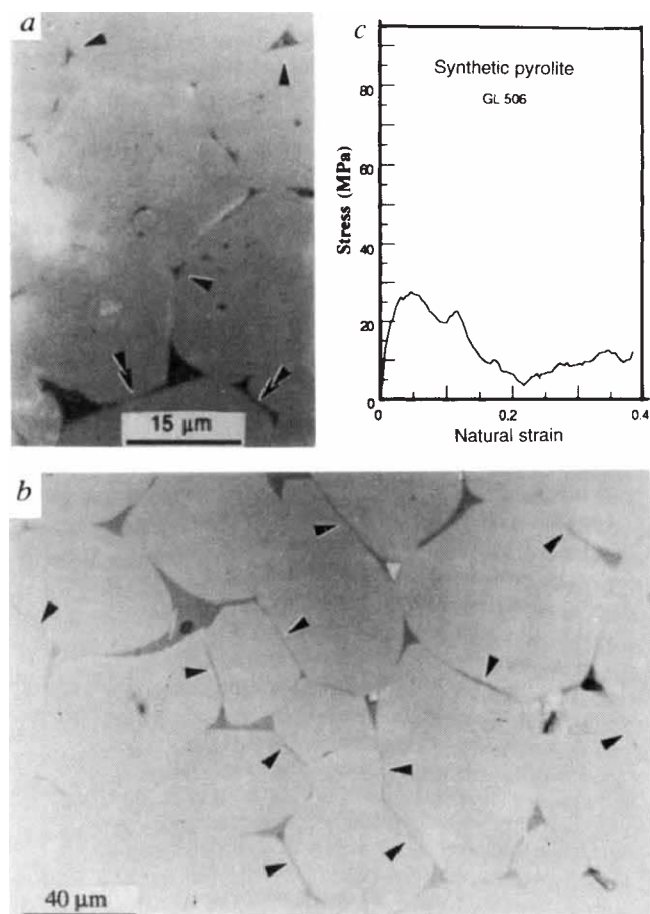


FIG. 1 *a*, Static microstructure: reflection optical micrograph of pyrolite specimen GL-525, annealed 40 h at 1.5 GPa, 1,475 K, showing equilibrated microstructure consisting of polygonal crystals and melt tubules of triangular cross-section along three-grain intersections (arrowheads). Note that near the bottom of the figure two flat wetted facets (double arrowheads) have developed, similar to the experimental results of Waff and Faul⁹. Except for these two boundaries, only one other boundary in this micrograph clearly has melt on it. *b*, Dynamic microstructure: reflection optical micrograph of pyrolite specimen GL-469, preannealed 12 h at 1.5 GPa, 1,500 K and shortened 41% at $3 \times 10^{-5} \text{ s}^{-1}$; boundaries generally are gently curved, and partial or complete melt films can be seen on ~80% of them (a representative sample is indicated by arrowheads); of 57 grain boundaries identifiable on this micrograph, 45 definitely have melt on them. Some of the others probably have films too thin to resolve optically. Maximum compressive stress in the north-south direction. *c*, Stress-strain curve for pyrolite specimen GL-506, preannealed 13 h at 1.5 GPa, 1,475 K, deformed at the same conditions to a total shortening of 32%, quenched under load to a temperature of 900 K and then returned to room temperature and pressure over 3 h.

Cylindrical specimens 3 mm in diameter and 7 mm long were cored from the synthetic rocks and dried for >100 h at 383 K. After specimen encapsulation in platinum, entire assemblies were stored in a vacuum oven at 383 K for ≥ 48 h before deformation in molten salt in a modified Griggs-type apparatus^{19,20}. Both annealing (12–40 h) and constant-strain-rate experiments were conducted at $P=1.5$ GPa and $T=1,475$ –1,500 K, yielding 4–7% melt. All deformed specimens were preannealed for 12–14 h at the run P and T to assure that they began with an approximately equilibrated microstructure.

Static annealing just above the solidus results in a melt distribution in which the most prominent feature is melt tubules of approximately triangular cross-section along three-grain intersections^{5–9} (Fig. 1*a*). By contrast, deformation at 10–40 MPa differential stress after such annealing results in a microstructure in which numerous triangular triple junctions are still present, but the majority of grain boundaries now exhibit melt along all or part of their length (Fig. 1*b*). During flow, the stress initially rises to a larger value and then falls off to a quasi-steady lower value (Fig. 1*c*).

To investigate the difference between dynamic and static experiments, we re-encapsulated half of one deformed specimen (GL-506), and reannealed it as experiment GL-509 for 40 h. If the differences between static and dynamic experiments were trivial or reversible, the second anneal should have yielded microstructures similar to those in Fig. 1*a*. On the contrary, the melt films established on grain boundaries during deformation were not removed by annealing. Rather, they broke up into rolls and droplets (Fig. 2*a, b*) characteristic of healing of thin films^{23–25} due to surface-energy minimization^{26,27}. Specimen GL-506 was deformed for only 4 h, representing 10% of the subsequent annealing time as experiment GL-509. Nevertheless, a large fraction of grain boundaries became covered with melt during the 4-h deformation, whereas those same melt films had only begun to re-equilibrate at the end of the 40-h reanneal. Spreading of melt onto grain boundaries clearly occurs much faster than its removal.

Evidence of grain-boundary migration is prominent in all specimens deformed in the presence of melt: (1) the grain size approximately doubles during 4 h of deformation (Fig. 1); (2) grain boundaries are curved and irregular, and indentations of one grain into another are common (arrowheads, Fig. 2*d*); (3) cusps are common on grain boundaries (double arrowheads, Fig. 2*c, d*), indicating local retardation of boundaries. By contrast, reannealing after deformation produces no change in grain size after 40 h (compare Fig. 1*b* with Fig. 2*a, b*).

To investigate the mechanism of deformation in the solid matrix, we oxidized some of our specimens to decorate the dislocations in olivine^{28,29} (Fig. 2*c*). Olivine crystals have a remarkably homogeneous distribution of free dislocations, and subgrain boundaries in various stages of formation, indicating that intracrystalline plasticity is an important component of the flow process. Most grain boundaries, including those between pyroxene grains (that do not themselves oxidize), are covered with oxidized, partially devitrified, glass. Dislocation densities (ρ) varied with the stress supported by the specimens. (For example, in GL-506: flow stress, 10 MPa; $\rho=(5\text{--}8) \times 10^6 \text{ cm}^{-2}$. In GL-502: flow stress, 30 MPa; $\rho=(1\text{--}2) \times 10^7 \text{ cm}^{-2}$.)

These results demonstrate that in partially molten mantle rocks deforming under low stresses in which dislocation creep is occurring in the solid phases, the melt distribution is different from that developed during static annealing. It is clear that spreading of melt onto grain boundaries is a dynamic effect; otherwise, the reannealed specimen would not have shown melt topology starting back toward microstructural equilibrium. This behaviour (fluid in triple junction tubes under static conditions, in grain-boundary films during deformation, and roll/bubble formation during reannealing) is identical to that found in salt-brine experiments^{12,16,30}. Stress-induced wetting of grain boundaries has also been recognized in feldspar³¹.

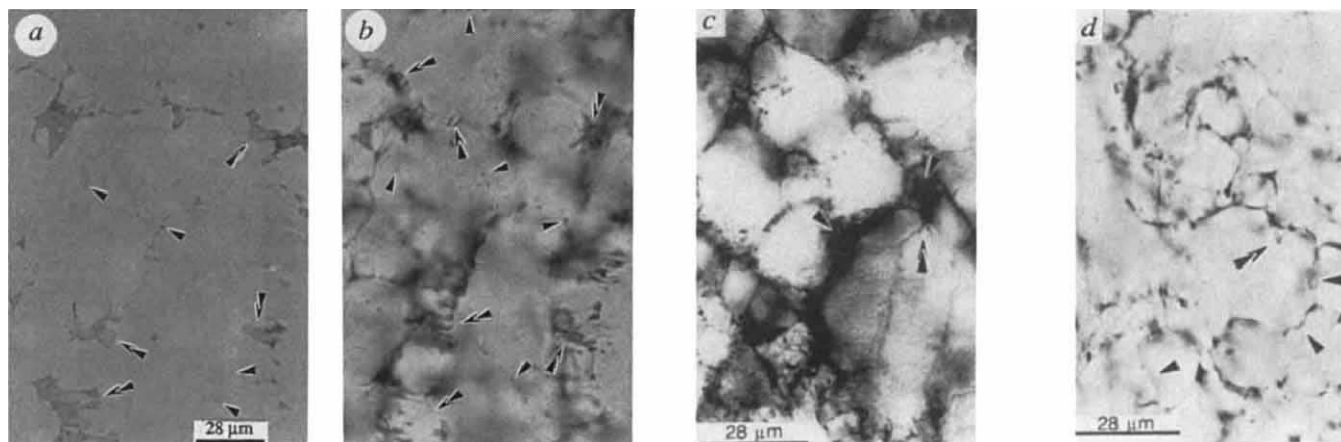


FIG. 2 *a, b*, Reannealed microstructure: pair of optical micrographs, at the same magnification (reflection, left; transmission, right) showing distribution of melt in specimen GL-509 (half of specimen GL-506) that was annealed for 40 h at 1.5 GPa, 1,475 K after deformation. Parallel striations (double arrowheads) and spots (arrowheads) abundant on grain boundaries are tubes and droplets of melt that have developed as the melt films on grain boundaries have begun to decompose in response to surface-energy forces. *c*, Optical micrograph of a thin section of an oxidized portion of specimen GL-502 showing a very uniform density of dislocations (ρ) in olivine ($\rho = (1-2) \times 10^7 \text{ cm}^{-2}$) and oxidation

of melt on grain boundaries. A highly irregular, melt-filled boundary of a large olivine crystal displays a cusp (double arrowhead) where it is retarded in completing its consumption of another olivine grain (arrowheads). Pyroxenes do not oxidize and therefore appear clear, but their boundaries, like the olivine boundaries, are decorated by iron oxides in the partially devitrified glass. *d*, Optical transmission image of a thin section of specimen GL-506 showing irregular grain boundaries with cusps (double arrowhead) and indentations (arrowheads) that are characteristic of dynamic partial melting. Maximum compressive stress in the north-south direction for *c* and *d*.

We do not yet fully understand the reasons for disequilibrium spreading of melt onto grain boundaries during low-stress creep. The drop in stress after flow begins (Fig. 1c) suggests that the static microstructure (melt primarily in triple-junctions) is stronger than the dynamic microstructure (melt on grain boundaries). Combined with the observed enhancement of grain boundary migration in our dynamic experiments and those on wet salts, the stress-strain behaviour suggests that the fluid reduces resistance to flow by enhancing grain-boundary sliding accommodated by dissolution in regions of stress concentration.

It is difficult to determine *a posteriori* from published micrographs whether or not dynamic wetting has occurred but gone unnoticed in previous studies of partial melting of mantle rocks or their analogues. Cooper and Kohlstedt¹¹ show a single micrograph of a specimen with a low degree of melting that shows no significant difference from our static specimens or the studies of Waff and colleagues^{5,8}. On the other hand, the specimens of Bussod and Christie¹⁸ show a definite tendency toward departure from triple-junction melt tubes (manifested at least in part by the 'slots' of melt induced by high stresses), as do those of Beeman and Kohlstedt¹⁷. Indeed, the micrographs in ref. 17 closely resemble ours. This perhaps is not surprising in that the latter study, like ours, involved dislocation creep with 4–8% melt at stresses sufficiently low to avoid microcracking, whereas that of Cooper and Kohlstedt^{10,11} involved diffusion creep.

Although it is unlikely that the stresses in our experiments are as low as those present in the mantle during partial melting, they are comparable to (or lower than) those inferred from the microstructures of normal mantle xenoliths from the oceanic upper mantle³² (not to be confused with high-stress mantle xenoliths deformed during the extraction process^{32–34}). This indicates that our experiments were conducted under stresses comparable to those responsible for flow during formation of the lithosphere. It is likely that flow deeper beneath ocean ridges also is characterized by dislocation creep in the solid residuum, as underlined by the recent discovery of pronounced seismic anisotropy directly beneath the Mid-Atlantic Ridge, with the vertical velocity greater than the horizontal³⁵. The only known mechanisms for producing anisotropy of this orientation and magnitude are preferred vertical alignment of olivine *a*-axes or preferred

vertical orientation of melt-filled cracks. The former develops only during dislocation creep, whereas microcracking develops only at stresses sufficiently high that the matrix is flowing by dislocation creep. Either scenario, therefore, suggests that the dislocation-creep, partially-molten flow regime sampled by the experiments discussed here corresponds to that present during upwelling beneath spreading centres, in agreement with studies of fossil spreading centres preserved in ophiolites³⁶. In addition, 'orientation families' (amoeboid and disconnected portions of the same crystal) observed to develop during extensive dynamic recrystallization of brine-saturated salts¹² have also been found in mantle xenoliths from the oceanic lithosphere (J. Urai and H.W.G., unpublished data), strengthening the evidence that dynamic wetting of peridotite grain boundaries by basaltic melt occurs in nature as well as in experiments.

The effective viscosity of these partially-molten specimens ($\sigma/\dot{\epsilon}$, where σ is stress and $\dot{\epsilon}$ is strain rate) is very low, suggesting that flow of the residuum during melt extraction could be significantly easier than implied by olivine rheology at the same temperature, so long as a small amount of melt remains behind. This effect could contribute to the separation of melt and residuum at very low melt fractions, as inferred from the trace-element compositions of ridge basalts³⁷. That is, in the Earth, melt may be extracted as soon as its abundance reaches the level where it spreads onto grain boundaries, restricting melt fractions in nature to values below those found in dynamic studies. Our preliminary mechanical data^{19,20} also suggest a relatively large effect of melting on flow stress. These observations suggest that in the Earth, spreading of melt onto grain boundaries could participate significantly in melt extraction and in enhancement of upwelling very close to spreading axes³⁸. □

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A balanced cross-section of the 1994 Northridge earthquake, southern California

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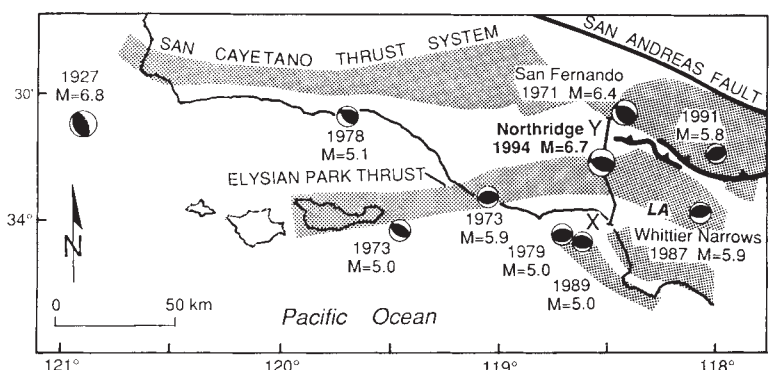
THE Northridge earthquake of 17 January 1994¹ was the latest in a series of very damaging, thrust-fault-generated earthquakes to strike California, following the San Fernando² 1971, Coalinga³ 1983, and Whittier Narrows^{4,5} 1987 events. Like the last two of these, the Northridge event occurred along a fault that did not reach the surface and which had not been detected by traditional seismic-hazard methods^{6,7}. Balanced cross-sections^{8,9}, which flatten and remove the crustal deformation, can be used to identify and quantify the seismic hazard posed by thrust faults. Here we present a balanced cross-section through the Northridge portion of the Transverse Ranges fold-and-thrust belt¹⁰, which shows that the earthquake occurred on what we call the Pico thrust. A cross-section of this sort constructed before the earthquake would have revealed the fault, although it would not have predicted the earthquake. Cross-sectional modelling of the Pico thrust yields an average slip rate of $1.4\text{--}1.7\text{ mm yr}^{-1}$ and a recurrence interval of Northridge-sized (M_w 6.7) earthquakes every 1,500–1,800 years. We show that the Pico thrust is the back thrust to the 170-km Elysian Park thrust^{3,4} which underlies some of the most densely urbanized portions of the Los Angeles basin.

Belts of crustal convergence (fold-and-thrust belts), active and ancient, have been the focus of much study by structural geologists during the past 80 years¹¹ and developing techniques that can forecast the subsurface structure in these belts has considerable economic importance for the exploration of oil and gas reserves^{9,12}. These techniques have focused on methods of cross-section balancing which kinematically restore the crust to an undeformed state (following the classical mechanics principle of conservation of mass) to test the validity of the interpretation¹³. Balanced cross-section construction is further constrained by geometric and kinematic theories and field observations of the origin of fault-related folds. The geometry of folds is most important because it is a direct result of fault geometry and slip at depth and is the most commonly available and continuous data set in fold-and-thrust belts. The balanced cross-section and seismological data from the Northridge earthquake presented here indicate that the present crustal structure, developed over several million years, and the recent earthquake are the result of displacement along a common fault deep below the surface.

The Northridge earthquake occurred at a depth of 18.0 km on a previously unknown thrust fault (Fig. 1). The focal mechanism and aftershocks of the earthquake indicate that the causative fault dips $\sim 40^\circ$ to the south⁵ and that the upper plate moved northwards. The causative fault did not rupture to the surface¹⁴ although the Santa Susana Mountains were uplifted $\sim 380\text{ mm}$ (ref. 15).

Subsurface projection of surface geology¹⁶ and data from oil wells show that the earthquake occurred beneath the San Fernando Valley synclinorium which lies between the Santa Monica Mountains and Santa Susana Mountains anticlinoria (Fig. 2a, b). The northeast limb of the Santa Susana Mountains anticlinorium consists of a panel of beds 2–3 km wide, dipping 60° –

FIG. 1 Epicentres, focal mechanisms and local or moment magnitudes M of the Northridge 1994 earthquake⁵ and other significant thrust earthquakes of southern California. Stipple pattern shows the location of major thrust ramps in the western Transverse Ranges^{3,10} which have been the source of many of the significant thrust earthquakes. LA, downtown Los Angeles; X–Y, line of cross-section in Fig. 2.



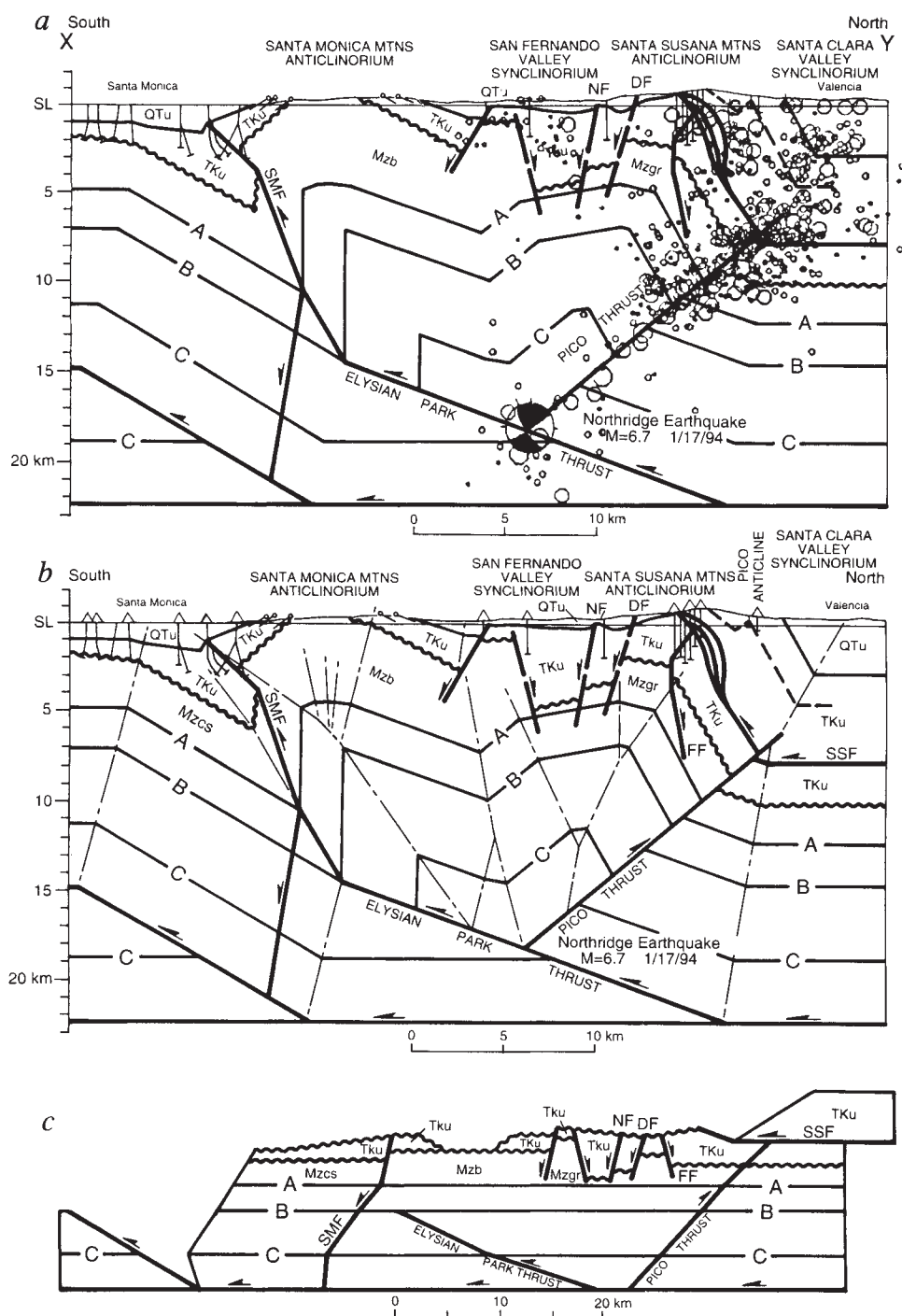
70° to the north. Field mapping following the earthquake showed activation of a discontinuous set of bedding-plane faults with slip directions consistent with coseismic growth of the anticlinorium¹⁷. Integration of deep oil-well data reveals the enormous vertical dimensions of the anticlinorium which has more than 6 km of structural relief (Fig. 2b). The gently dipping south limb shows that the anticlinorium is asymmetric to the north.

Coseismic uplift of the Santa Susana Mountains anticlinorium and surface disruptions characteristic of folding¹⁷ indicate it is a fault-related fold. We interpret the Santa Susana Mountains anticlinorium to be a fault-propagation fold¹⁸ based on its asymmetric geometry. In this type of thrust-related fold, an asymmetric anticline forms above a propagating thrust ramp with fault slip terminating in the synclinal axis in front of the fold (Fig.

2b). We refer to the causative fault of the Northridge earthquake and the Santa Susana Mountains anticlinorium as the Pico thrust, because the large Pico anticline occurs as a secondary structure along the northeast limb of the anticlinorium¹⁶. This interpretation also provides a kinematic explanation of the zone of shallow aftershocks as flexural-slip faulting, fold axis migration into the Santa Clara synclinorium, and incipient propagation of the Pico thrust up the synclinal axis (Fig. 2a). These secondary processes are commonly observed in the front limbs of fault-propagation folds¹⁸ and are consistent with the discontinuous surface disruptions observed in the Santa Clara synclinorium^{14,17}.

The steep north limb and gentle south-dipping back limb of the anticlinorium requires a south-dipping thrust, but the back limb only constrains the dip of the fault to a range of 25°–45°

FIG. 2 a, Balanced cross-section across the Northridge portion of the Transverse Range fold-and-thrust belt with focal mechanism⁵ and aftershock distribution⁵ from the Northridge 1994 earthquake superimposed. Main event and aftershocks below 5 km occurred along the Pico thrust. Shallower aftershocks are the result of fold growth (flexural-slip faults) and propagation of the fold hinge into the Santa Clara synclinorium. Thick black lines show faults, wavy lines show unconformities. b, Balanced cross-section showing the Santa Susana Mountains and Santa Monica Mountains anticlinoria as crustal-scale fault-propagation folds above the Pico and Elysian Park thrusts, respectively. Assuming that the Northridge earthquake is the characteristic event for the Pico thrust, then it would take ~1,300 events to make the present-day Santa Susana Mountains anticlinorium. Location of wells are shown (triangles) with bore holes, and long-short dashed lines are fold hinges. c, Restoration of the cross-section shows that it balances and is therefore a viable solution¹³. The Santa Monica Mountains and Santa Susana Mountains anticlinoria are unfolded, and slip on the Elysian Park and Pico thrusts is restored to the pre-growth setting (~2–3 Myr ago). High-angle faults are older Miocene- and Pliocene-age normal faults that formed before regional convergence. Abbreviations: NF, Northridge Hills fault; DF, Devonshire fault; FF, Frew fault; SSF, Santa Susana fault; SMF, Santa Monica fault; QTu, Upper Pliocene and Quaternary rocks; TKu, Lower Pliocene to Upper Cretaceous rocks; Mzcs, Catalina Schist; Mzb, Mesozoic age metamorphic and plutonic rocks; Mzgr, Mesozoic age granite, A, B and C, form lines showing Late Cenozoic convergent structure in the undifferentiated crystalline basement.



and only generally constrains the fault depth. Integration of seismological data on the hypocentre, mainshock focal mechanism and distribution of aftershocks establishes the exact dip and depth of the Pico thrust.

Our interpretation indicates a pairing of the Santa Susana Mountains anticlinorium uplift and the deeply buried Pico thrust. The lateral extent of the latter can therefore be defined by the lateral extent of the Santa Susana Mountains anticlinorium which is recorded by surface geology and subsurface well data to be 30–40 km in length. More work is needed to elucidate the detailed geometry of the anticlinorium to define more precisely the regional geometry and extent of the seismically active Pico thrust.

The Pico thrust is shown to be a backthrust off the north-dipping Elysian Park thrust ramp. The Elysian Park thrust is rooted in a mid-crustal detachment at ~22 km depth. Mid-crustal detachments have been proposed by numerous workers^{3,10,19–21} to underlie the western Transverse Ranges and are a basic component of fold-and-thrust belts^{8,9,13}. The restored cross-section (Fig. 2c) shows the structural geometry before folding and faulting, and provides a check that the cross-section is balanced.

The balanced cross-section provides information about the seismic potential of the Pico and Elysian Park thrusts. This information is especially important for the latter thrust because it underlies the most urbanized parts of the Los Angeles basin (Fig. 1). Previous balanced cross-section analysis and subsurface mapping of the Elysian Park thrust^{3,22} indicate that it is over 170 km long and is a fundamental thrust fault of southern California. The Pico thrust has 3.3 km of displacement and the Elysian Park thrust has 11.8 km of displacement (Fig. 2b). Compressive deformation probably began 2–3 Myr ago³ within the Transverse Range fold-and-thrust belt. Initiation of the Santa Susana Mountains anticlinorium and Pico thrust is recorded by deformation of the youngest unit, the Saugus Formation (QTu, Fig. 2), which is no older than 2.3 Myr (ref. 23). The displacement and 2.3–2.0 Myr age of fault initiation yields an average slip rate of 1.4–1.7 mm yr⁻¹ for the Pico thrust. A 2.0–3.0 Myr (ref. 3) age of initiation of the Santa Monica Mountains anticlinorium yields an average slip rate of 3.9–5.9 mm yr⁻¹ for the Elysian Park thrust.

Geodetic and seismic modelling suggest the Pico thrust moved ~2.5 m during the Northridge earthquake^{15,24} which, divided by our slip rates, yield an average repeat time of 1,500–1,800 years. This repeat-time estimate applies to the segment of the Pico thrust involved in the earthquake, which is ~15 km long. Similar recurrence calculations can be made for the Elysian Park thrust (Fig. 1). Assuming that a Northridge-size earthquake is the characteristic event for the Elysian Park thrust trend, then our slip rate and time-of-initiation estimates yield average earthquake repeat times of 420–640 years for any 15 km segment of the thrust, or an event every 39–58 years along the trend. This repeat time is not supported by the 220 years of recorded history. This discrepancy is probably due to one or more of the following; our long-term slip rates differ from the short-term rates, some crustal shortening is taken up aseismically, the earthquake repeat time is variable, or the characteristic event is even larger and less frequent than that predicted by a Northridge-size earthquake.

Fold-and-thrust belts are tectonic systems with complicated fold and fault patterns that develop over a scale of kilometres (ref. 13). In seismically active belts, the integration of earthquake data with balanced cross-sections provides a broader view of seismic risk than traditional seismic-hazard methods. Application of the balanced cross-section technique should be the first step in evaluating the seismic risk in fold-and-thrust belts and would have predicted the presence of a young south-dipping thrust beneath the Santa Susana Mountains and San Fernando Valley before the Northridge earthquake. □

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Symmetry, beauty and evolution

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HUMANS and certain other species find symmetrical patterns more attractive than asymmetrical ones. These preferences may appear in response to biological signals^{1–3}, or in situations where there is no obvious signalling context, such as exploratory behaviour^{4,5} and human aesthetic response to pattern^{6–8}. It has been proposed^{9,10} that preferences for symmetry have evolved in animals because the degree of symmetry in signals indicates the signaller's quality. By contrast, we show here that symmetry preferences may arise as a by-product of the need to recognize objects irrespective of their position and orientation in the visual field. The existence of sensory biases for symmetry may have been exploited independently by natural selection acting on biological signals and by human artistic innovation. This may account for the observed convergence on symmetrical forms in nature and decorative art¹¹.

It is striking that many signals used for communication by organisms are judged to be beautiful by humans. Examples include the colours and symmetries of flowers, the patterns on butterflies' wings and coral reef fish, and the elaborate courtship displays of birds. The almost universal appeal of such signals to humans is surprising because they have been selected to influence the behaviour of animals whose visual systems differ in significant ways from our own, and which in some taxa (for example insects and cephalopods) have evolved independently¹². This raises the possibility that human aesthetic sense is based on general principles of perception that have been important during the evolution of biological signals.

Studies in early ethology¹³ and experimental psychology^{14,15}, have shown that sensory systems react strongly to certain novel stimuli. Such biases impose a selection pressure on signals^{16–18}, causing them to become more extreme in form and polarized during evolution^{19,20} (typically larger, louder, brighter and otherwise different from stimuli that the receiver should not react to in the same way). However, it is less obvious that the striking

degree of patterning seen in signals, and preferences for such patterns, are also the consequences of sensory bias.

One problem faced by animals is the need to recognize objects in different positions and orientations in the visual field. An object viewed from a particular location is focused on the retina as an 'image', which is a geometrical transformation of the object itself. An intriguing idea is that the need to generalize many such transformations of the same object may lead to preferences for symmetry²¹ and the evolution of symmetrical signals. To explore

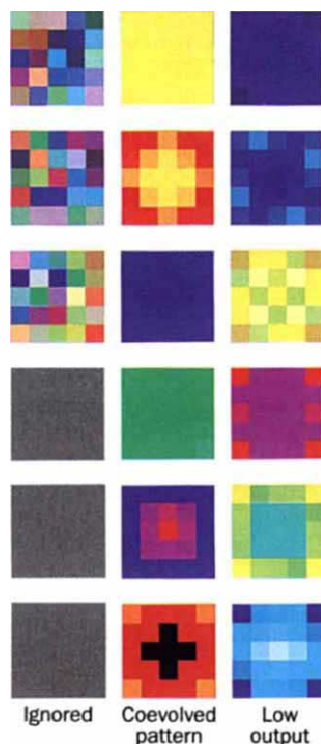


FIG. 1 Symmetry generated by coevolution between a visual signal and a recognition mechanism. Results of six independent simulations are shown, one in each row. Signals consisted of coloured squares in a 5×5 grid, each square containing a single colour. The recognition mechanism was an artificial neural network with 147 input cells, 15 hidden cells and one output cell. Each cell in a given layer was connected to all cells in the next layer, but cells within the same layer were not interconnected. The input cells were arranged on a square retina with 7×7 locations, each location containing 3 input cells sensitive to a different part of the colour spectrum according to the RGB system²². Each input cell receives stimulation between 0 and 1 corresponding to the degree of saturation of the respective colour element. A random pattern was generated and designated as the 'correct' signal, and an alternative pattern (either random or uniform grey) was designated as the pattern that the network should ignore. The patterns were pasted onto the retina in various positions by translation and rotation, and the response of the network measured. A network was said to react to a signal when the activity in its output cell plus a random internal factor (normally distributed with zero mean, s.d. 0.02) exceeded a threshold value of 0.5. Initially, connection weights in each network were randomly assigned, varying between -0.3 and 0.3 in a rectangular distribution. A simulation consisted of 500 generations, each involving 100 mutations of both the signal and the network. Mutations of a network were produced letting each connection weight mutate with probability 0.01 and adding a normally distributed increment (zero mean, s.d. 0.02) to each mutated weight. Mutations of the signal were produced by mutating each colour element with probability 0.05, then adding (where possible) a normally distributed increment (zero mean, s.d. 0.3) to each mutated element. In each generation the best signal and best network were kept. The low output signals are those signals which tended to elicit the lowest responses from the coevolved networks when the connection weights held constant.

this idea we have simulated the coevolution of signals and receiver preferences, using artificial neural networks as models of recognition systems.

The technique used in simulations was similar to that described in ref. 19 (see also Fig. 1). Images of patterns ('signals'), consisting of coloured squares in a grid, were 'pasted' onto an artificial retina in various positions and orientations, mimicking some of the ways in which animals may see objects in the visual field. Starting with an arbitrary signal and arbitrary network (randomly assigned colours and connection weights), coevolution proceeded, in each generation, by producing a series of mutations of the signal and the network and retaining the best one of each (artificial selection). The criterion for selecting the signal from a series of mutations was that it should elicit the highest output from the network; the criterion for selecting the network was that it should discriminate between the correct sig-

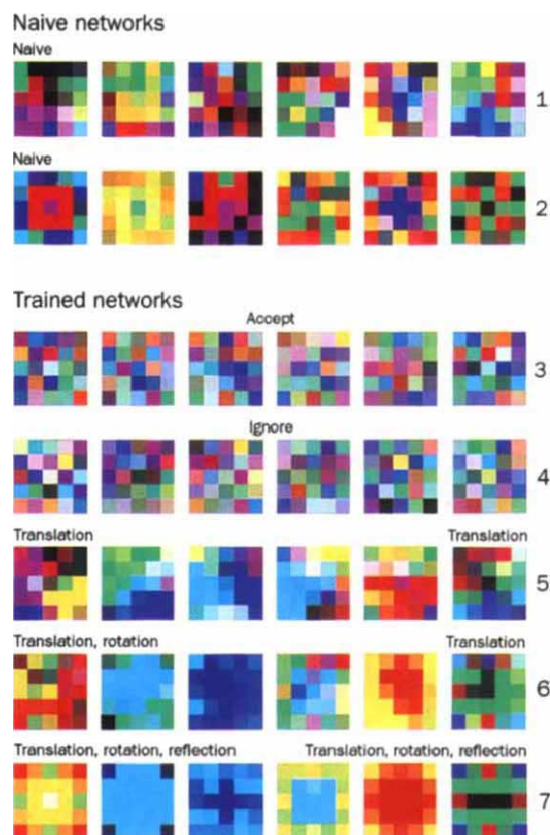


FIG. 2 The effect of different training and test procedures. The network training procedure is indicated on the left-hand side and the test procedure on the right-hand side above each row of signals. In all examples, the networks were first prepared and then connection weights held constant to explore their preferences. For six different 'naive' networks (randomly assigned connection weights), preferences are shown for signals projected onto the retina either by translation (top row, 1) or by a combination of translation and rotation (row 2). Six trained networks were prepared by teaching them to distinguish between two sets of random patterns that lack symmetry (rows 3 and 4). Training was done by projecting these patterns under translation only (row 5), translation + rotation (row 6), or translation + rotation + reflection (row 7) and continued until recognition errors were negligible ($<10^{-5}$). After training, preferences were tested under translation only (rows 5 and 6), or using all transformation types (row 7). In all cases, the preference test involved generating a signal that elicited a strong response in the network by repeated mutation and selection of signals for 500 generations (for details, see legend to Fig. 1). Signals shown directly under one another in each column are derived from the same starting network.

nal and a series of alternative patterns (Fig. 1: Ignored) with least error. This procedure eventually leads to a quasi-stable situation in which the network discriminates almost perfectly between correct and incorrect signal patterns and the signal itself (Fig. 1: Coevolved pattern) changes only slowly. After completing the simulation, the connection weights of the network were held constant, and signals were found that tended to minimize output (Fig. 1: Low output).

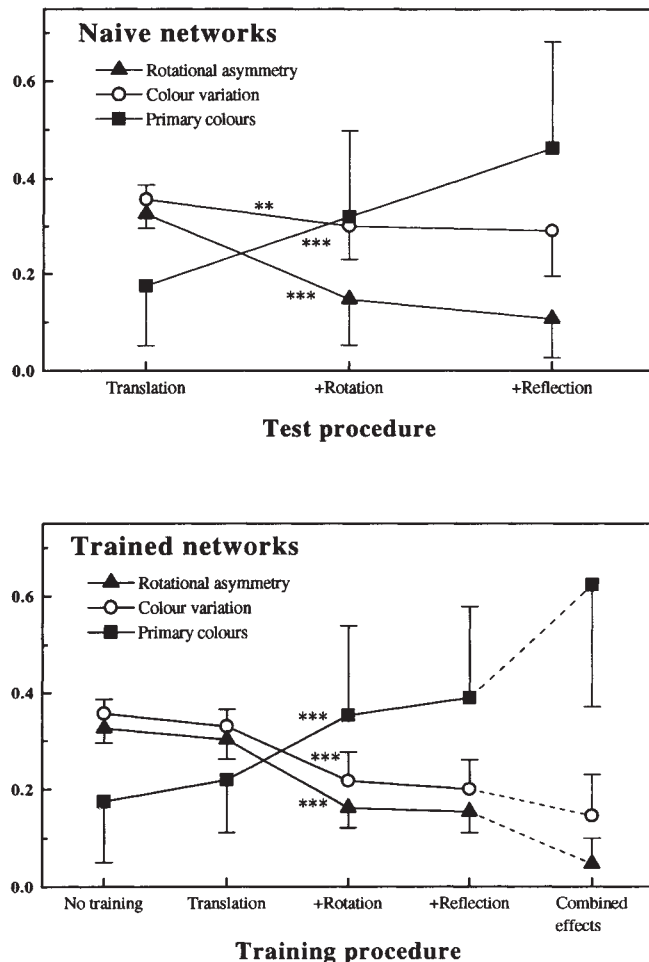


FIG. 3 Characteristics of preferred signals as a function of signal selection and network training procedures. Each point is based on 50 independent calculations. The bars represent standard deviations and the asterisks denote significant differences in comparison with preferred patterns generated by translation only. ANOVA was used except for test on primary colours which used Mann-Whitney *U*-test ($***P < 0.001$, $**P < 0.01$). Colour variation and rotational asymmetry were measured as the root mean square within groups. For colour variation, the groups were made up of the three RGB colour elements. For rotational asymmetry, groups were based on both colour element and position (3×6 groups). Under 90° rotation around the centre, a perfectly symmetrical pattern requires the colour to be identical at four locations (for example the four corners) within 6 different groups (excluding the central point). Primary colour is a measure of the relative frequency of such colours in the pattern. Any RGB triplet was considered to be a primary colour if none of its three elements deviated by more than 0.1 from zero or one. The x-axis shows differences in the method of projecting signals onto the retina during the test procedure (for naive networks) or during the training procedure (for trained networks). Preferred signals of trained networks were generated by selection of signals under translation only, except for one treatment (far right) which shows the combined effect of training networks and testing their preferences using all possible transformations permitted by the experimental design (translation + rotation + reflection).

The coevolved signals consisted of purer, brighter colours than random patterns; they also displayed significantly lower variation in colours, and marked symmetries. Signals producing a low output also displayed strong symmetries, but consisted of the chromatic opponents²² of those colours present in the coevolved signals. These results demonstrate polarization towards brighter colours in the signal, that is, a divergence from the 'ignored' patterns. They also show that signals least likely to provoke a response in a given situation are 'opposite' in appearance to those that cause a strong response (Darwin's principle of signal antithesis²³).

There are two possible causes for the symmetries that appeared during our simulations. Symmetry may result directly from selection acting on signals, irrespective of the state of the network. This is because a signal that, on average, is best at stimulating the network in all possible positions and orientations may take a symmetrical form. Alternatively, the network itself may become more sensitive to symmetry as a result of the evolutionary process.

To distinguish between these possibilities, we did two further tests (Figs 2 and 3). First, a random network was tested to find the signals that were most stimulating under different projection regimens (connection weights of the network were held constant). The degree of symmetry that developed in signals was found to depend strongly on the method of projecting the signals onto the retina during tests. For example, when signals were projected in all possible orientations, stronger rotational symmetries developed than when signals were projected by translation alone.

Second, networks were trained to recognize random, asymmetrical signals projected onto the retina in different ways (the signals were held constant). The preferences which then emerged depended strongly on the method of training the network. Networks trained to recognize random signals, irrespective of their orientation, developed preferences for signals with strong rotational symmetries. By contrast, preferences for symmetry were weak when networks were trained to recognize signals projected onto the retina in different locations, but in a fixed orientation.

These results show that preference for symmetry is a consequence of the need to recognize signals irrespective of their position and orientation in the visual field. In more detail, this is due to the combination of two effects that reinforce each other during coevolution: one effect is independent of the state of the network, and the other becomes important when the network is trained or evolves.

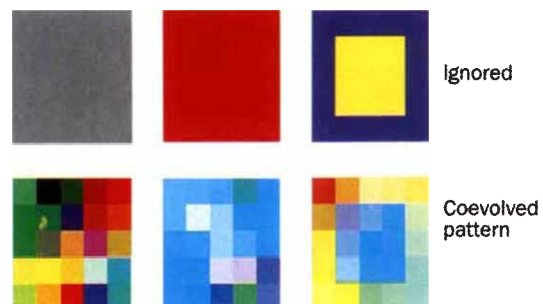


FIG. 4 The influence of the ignored pattern on the outcome of signal coevolution. Signals were selected under translation only. When the pattern to ignore was uniformly grey there are six equally likely directions of bias (red, green, blue, cyan, magenta, yellow) for each element in the coevolved signal: the precise outcome cannot be predicted. When the ignored pattern is uniformly red, the signal becomes polarized towards the blue end of the spectrum. When the ignored pattern contains more complex symmetries, the coevolved signal displays similar symmetry but in opponent colours.

Several alternative explanations exist for the occurrence of symmetrical signals and symmetry preferences in nature. It has been suggested that some morphological symmetries arise inevitably from developmental processes²⁴. However, as Wallace²⁵ observed, the symmetrical body markings of wild animals are often lost or degraded in their domesticated descendants. This suggests that certain symmetries are not inescapable consequences of development, but are maintained by other selection pressures in nature.

Preferences for symmetry observed in animals might reflect symmetries in the pattern of connection between nerve cells in the brain. Such preferences may also arise because many objects in the world that animals should distinguish are themselves symmetrical (see Fig. 4). However, whether symmetry is common enough in nature to make this important as a general explanation for symmetry preference remains unclear. Finally, it has been suggested that preferences for symmetry may have evolved for adaptive reasons connected with mate choice^{1,10}. Recent experiments on birds and insects have shown that females prefer to mate with males possessing the most symmetrical sexual ornaments¹⁻³. Because deviations from perfect symmetry are negatively correlated with fitness in some species⁹, the degree of symmetry in ornaments may provide females with information about male quality. But evidence that the amount of symmetry in ornaments provides females with information does not prove that preferences for symmetry evolved for that reason. Because such preferences emerge as a by-product of selection for recognition, any benefits that females gain by mating with symmetrical males may be best considered a fortuitous effect of sensory bias.

What implications, if any, do these findings have for aesthetics? Both humans⁶⁻⁸, and certain other species^{4,5}, find symmetrical patterns attractive in contexts unrelated to signalling. Such general preferences for symmetry serve no obvious function, but may result from the universal need among organisms to recognize objects irrespective of the manner in which they are encountered in the outside world. Our results suggest that in the process of learning to recognize objects, preferences can develop for particular forms that have no objective existence in nature. Such hidden preferences²⁰ are revealed only when the actual forms corresponding to them appear in nature, either through biological evolution or artistic innovation. □

Female preference for symmetrical males as a by-product of selection for mate recognition

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FLUCTUATING asymmetry (FA) refers to the random, stress-induced deviations from perfect symmetry that develop during the growth of bilaterally symmetrical traits^{1,2}. Individual differences in the level of FA may influence mate choice³: in a number of species, females prefer to mate with males that have more symmetrical sexual ornaments⁴⁻⁷. As the degree of FA has been shown to reflect the ability of individuals to cope with a wide variety of environmental stresses^{2,8,9}, it has been suggested that mating preferences for symmetry evolve for adaptive reasons, because the degree of FA provides honest information about male quality^{10,11}. Here I use simple, artificial neural networks to show that such preferences are likely to arise in the absence of any link between symmetry and quality, as a by-product of selection for mate recognition.

Female preferences for symmetrical male displays have been demonstrated in a number of species, including swallows^{4,5}, zebra finches⁶ and earwigs⁷. These preferences have commonly been explained by reference to the honest advertisement hypothesis or handicap principle¹². Many sexual ornaments are thought to be expensive to produce, and are under strong directional selection. Both of these factors lead to stresses that reduce the effectiveness of developmental homeostatic mechanisms. Consequently, the level of FA in male secondary sexual traits may provide females with honest information about the developmental competence of a potential mate^{3,10,11}. In support of this suggestion, there is evidence that the level of FA in some species is heritable and/or negatively correlated with one or more fitness measures such as viability, fecundity, and growth rate^{2,8,9,13,14}. However, evidence that FA currently provides females with useful information regarding male quality does not prove that preferences for symmetry initially evolved for that reason.

Here I test the hypothesis that preferences for low levels of FA in males may evolve in the absence of any link between symmetry and quality, as a by-product of selection for mate recognition. I investigate the evolution of symmetry biases by examining the properties of simple, artificial neural networks trained (by a process of artificial selection) to recognize a set of patterns that exhibit varying degrees of FA. Such networks, when used in the study of sexual selection and signal evolution^{5,16}, provide a way to examine general properties of recognition systems.

A network, representing the recognition system of a female bird, was 'trained' by artificial selection to recognize a suite of images representing a bilaterally symmetrical tail that exhibits varying degrees of FA (Fig. 1). This involved repeated mutation of the network to generate variants that differed slightly in their response to different patterns, and selection of those variants that tended to respond more strongly to the images in the training set than to random patterns. The training process thus simulates the effects of selection for mate recognition, in that it favours the ability to distinguish a specific (bilaterally symmetrical) display from other stimuli. Two different sets of training images were used: the first comprised all five of the patterns shown in Fig. 1, and the second comprised only patterns 1, 2, 4 and 5. Networks trained using the first set were thus exposed to the perfectly symmetrical tail pattern 3 during the selection procedure, whereas networks trained using the second set were not. Neither of the two training procedures gave any explicit

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advantage to network variants that favoured symmetrical over asymmetrical tail patterns. For each individual network, mutation and selection were repeated 50 times, leading to a gradual increase in the ability to distinguish tail patterns from random patterns.

To investigate whether selection for recognition gave rise to preferences for low levels of FA, I compared the response of networks to the five different tail patterns both before and after selection. If the recognition system represented by the networks was unbiased with respect to symmetry, it was predicted that each of the five tail patterns would be preferred with equal probability. Figure 2 shows the proportion of 1,000 networks that preferred each pattern (that is, responded more strongly to that pattern than to any other) at the start and at the end of training with the full set and with the partial set of tail patterns. After the first selection step, networks were no more likely to prefer one pattern than another ($\chi^2=2.78$, d.f. = 4, $P=0.5949$ for the full training set; $\chi^2=0.51$, d.f. = 4, $P=0.9728$ for the partial set). After the full selection procedure (involving 50 selection steps) had been carried out, however, the networks' preferences differed significantly from random ($\chi^2=138.1$, d.f. = 4, $P=0.0001$ for the full training set; $\chi^2=156.36$, d.f. = 4, $P=0.0001$ for the partial set), with more symmetrical tail patterns favoured over less symmetrical patterns. The perfectly symmetrical image 3 was the most frequently preferred, even by networks that had been trained using the partial set of tail patterns, and had not therefore been exposed to pattern 3 during training. The preference for symmetry in tail patterns did not, however, extend to symmetry in other images; investigation of the networks' responses to randomly generated patterns not included in either training set frequently revealed hidden preferences for asymmetrical shapes.

To examine the interaction between preferences for symmetry and preferences for greater elaboration of ornaments, I compared the networks' responses to a large set of tail patterns that were similar to those in the training set but which differed in length as well as symmetry. Figure 3 shows which of these patterns were favoured over others for networks trained using the full set of images shown in Fig. 1, and reveals that the training procedure gave rise to preferences for longer tails as well as more symmetrical tails. Differences in length between two tail patterns

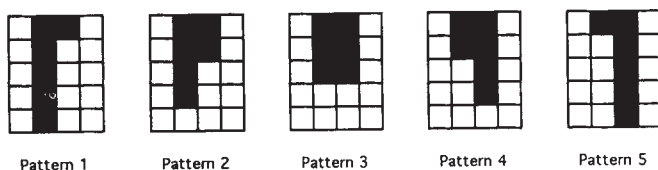


FIG. 1 Set of five tail patterns used to train networks. The neural networks themselves consist of three layers of cells: a 'retina' of 4×5 receptor cells, five hidden cells and one output cell. Each cell in a layer connects to all cells in the next layer, and each connection is associated with a weight (ranging from -1 to $+1$), which regulates the strength of the signal passing between the two cells. When a network is presented with a pattern, each receptor cell receives an input of either 0 or 1. The output from these cells is equal to the input. The input to each of the hidden cells and to the output cell is a weighted sum of the outputs from all cells in the previous layer; the output from these cells is a sigmoid function of the input, specifically $(1 + \tanh(i/2))/2$, where i denotes the input. Networks were trained to discriminate between random images and either the full set of five tail patterns shown here, or a partial set comprising only patterns 1, 2, 4 and 5. Note that the number of receptor cells on the retina stimulated by each tail pattern is equal, so that any differences in the response to each are not due to differences in the overall amount of stimulation. Random patterns were also generated in such a way that while each receptor cell on the retina was equally likely to be stimulated by the pattern, the mean overall amount of stimulation was equal to that provided by these tail patterns. Furthermore, they were generated so that their mean level of symmetry was identical to that of the set of tail patterns.

could thus, on occasion, outweigh the effects of a difference in symmetry. For example, tail patterns with a mean length of three pixels that exhibited a low level of asymmetry were preferred to a perfectly symmetrical tail pattern with a mean length of two pixels ($\chi^2=89.08$, d.f. = 1, $P=0.0001$ and $\chi^2=158.61$, d.f. = 1, $P=0.0001$, for the two comparisons involved). A larger difference in the level of FA, however, outweighed the effects of the bias towards greater length; a symmetrical tail pattern with a mean length of two was preferred to tail pattern with a mean length of three pixels that exhibited a high level of asymmetry ($\chi^2=46.13$, d.f. = 1, $P=0.0001$, and $\chi^2=21.78$, d.f. = 1, $P=0.0001$ for the two comparisons involved). Although the preference for longer tails, like the preference for symmetry, extended to patterns not included in the training set, it was not open-ended. Comparing the networks' responses to symmetrical tails of different sizes, tail patterns with a mean length greater than three pixels (the length of the training images) were preferred

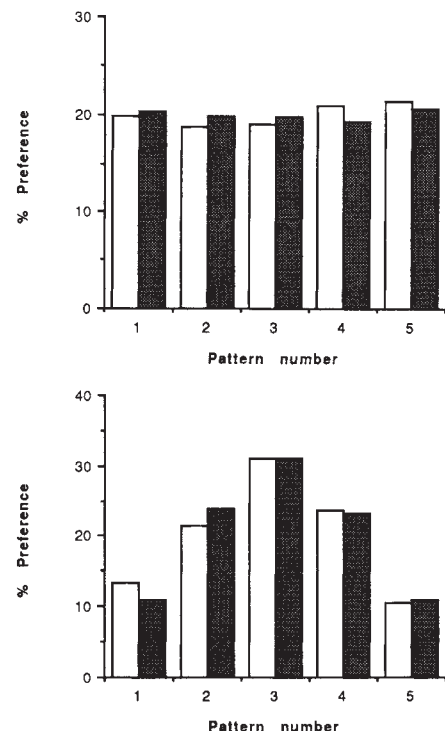


FIG. 2 Consequences of selection for recognition ability on preferences for symmetry. The selection procedure was as follows: starting with an original population of five networks each defined by a randomly generated set of weights, a new population was generated by selecting one member of the old, and replacing the other four with variants of this chosen network. The variants were created by a mutation procedure in which, with a probability of 0.05, each weight was replaced with a new, random value (evenly distributed between 0 and 1). The network chosen for replication was selected as follows: each member of the original population was scored by determining the mean value of its response to the patterns in the training set, and the mean value of its response to a set of randomly generated patterns, and subtracting the latter value from the former. The network with the highest score was then chosen. This process of mutation and selection was repeated 50 times. The selection procedure thus favoured those networks that responded more strongly to tail patterns than to a single random pattern generated at the start of the training procedure). The graph shows the proportion of 1,000 networks that preferred each tail pattern (that is, responded more strongly to that tail pattern than to the other tail patterns) after the first selection step (top) and after the last (bottom), for both the full and partial training sets (open and shaded bars, respectively).

to shorter patterns, but a tail of mean length four was preferred to one of mean length five ($\chi^2 = 58.98$, d.f. = 1, $P = 0.0001$).

The selection procedure probably gave rise to preferences for symmetrical tail patterns because such patterns are closer to the average of the training stimuli used. In both the full and partial training sets, the asymmetrical patterns formed mirror-reflecting pairs. As a result, the average of the training patterns, shown in Fig. 4, was itself symmetrical. Selection for recognition of the training set thus gave rise to preferences for tail patterns close to this symmetrical average. Symmetrical images that were randomly generated were not necessarily preferred, because they could diverge substantially from the average of the training stimuli.

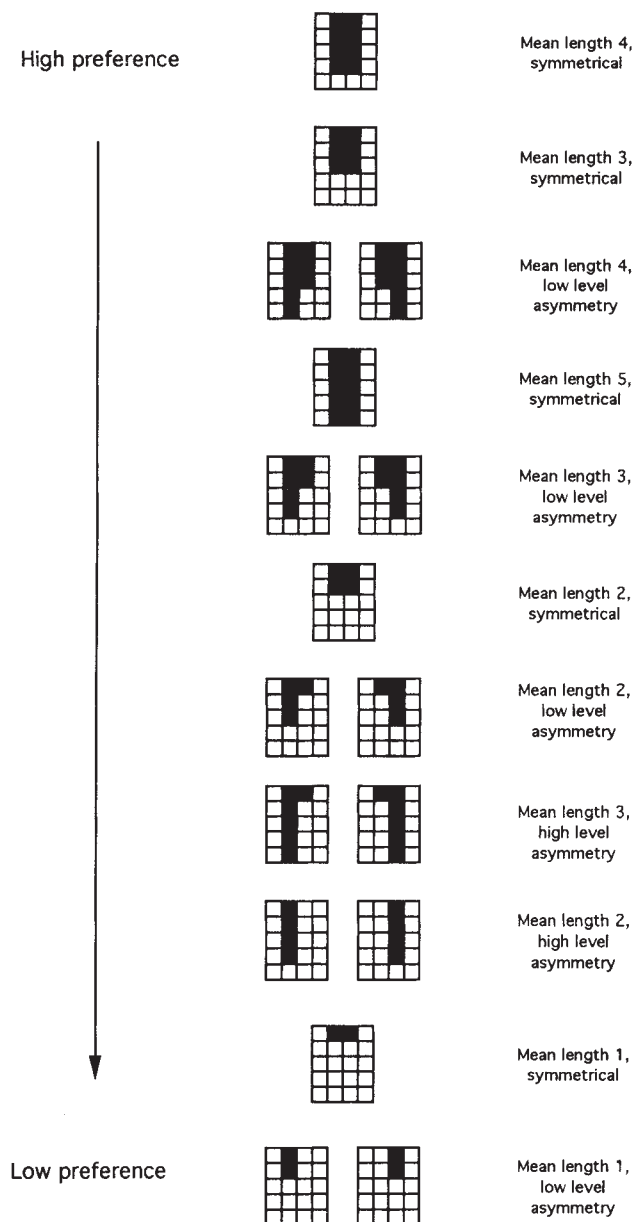


FIG. 3 Hierarchy of preference among tail patterns that vary in both length and symmetry, for networks trained using the full set of five patterns shown in Fig. 1. In a 2-way comparison, the proportion of networks that preferred any given pattern to another from the same or higher rank in the hierarchy is never significantly greater than expected by chance ($P < 0.05$). At the same time, there was a significant preference for any given pattern over at least one image from each lower rank in the hierarchy.

Preferences for longer tails probably evolved because long tail patterns overlap more of the training set average. Although all the training images comprised six filled pixels in total, and thus had a mean length of three pixels, the maximum length of the asymmetrical tail patterns was greater (Fig. 1), because asymmetry involves an increase in length of one side of the tail. As a result, the average of the training patterns, shown in Fig. 4, extends beyond the mean length of three pixels to the maximum length of the most asymmetrical training patterns. Selection thus gave rise to preferences for tails of greater than normal length, which overlap more of this average. The preference was not, however, open-ended, probably because the networks tended to penalize joint filling of pixels on either side of the midline in the bottom two rows of the pattern (an occurrence that was never reinforced by the training stimuli).

Paired ornaments in nature, such as swallows' tails and earwigs' forceps, exhibit symmetrical variability just as the training stimuli did (FA being defined as deviations from symmetry that are random with respect to side, and whose population average is therefore nil). Consequently, symmetrical ornaments are closer to the population average display. The above results thus indicate that when males of a species possess paired ornaments, selection for female recognition of appropriate mates can lead to biases favouring symmetrical males. Preferences for males with low levels of FA can therefore evolve even if there is no relationship between symmetry and mate quality, and no advantage to be gained by obtaining a symmetrical partner. Moreover, such preferences may extend to a degree of symmetry that females rarely or never encounter in natural populations.

Proponents of the 'sensory exploitation' hypothesis¹⁵⁻²¹ have argued that female preferences for elaborate male displays may

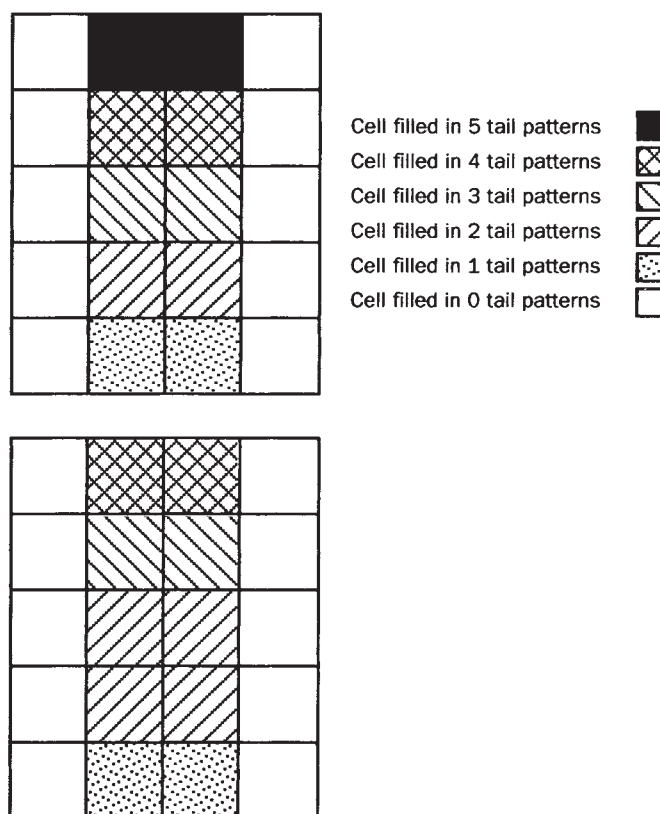


FIG. 4 Average of the training stimuli. In the case of both the full (top) and the partial (bottom) training sets, this average is symmetrical, and closer to the perfectly symmetrical tail pattern 3 than to any other tail pattern in the training set. It also extends beyond the mean length of the individual training stimuli.

often evolve for non-adaptive reasons, as a by-product of selection for other aspects of female perceptual abilities. Enquist and Arak¹⁵, for example, have demonstrated that such preferences can evolve as a by-product of selection for mate recognition. Similar explanations for symmetry biases have not, however,

been advanced. The present model shows that the sensory exploitation hypothesis is equally applicable to preferences for low levels of FA as to preferences for ornament elaboration, and that in species with bilaterally symmetrical displays, both types of preference are likely to evolve together. □

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Cell interaction between compartments establishes the proximal–distal axis of *Drosophila* legs

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THE appendage primordia of *Drosophila* are subdivided into compartments^{1–4} by the localized expression of transcription factors^{5–7}. Interaction between cells in adjacent compartments establishes organizing centres responsible for generating spatial pattern and promoting cell proliferation in the developing appendages^{7–9}. Localized expression of *hedgehog* (*hh*) in the posterior compartment of the leg imaginal disc directs expression of *wingless* (*wg*) in ventral–anterior cells and *decapentaplegic* (*dpp*) in dorsal–anterior cells near the anterior–posterior compartment boundary⁸; *wg* then acts to specify ventral cell fate^{10–12} and to organize the dorsal–ventral axis of the leg^{13,14}. Interaction between

wg-expressing ventral cells and dorsal cells near the anterior–posterior compartment boundary promotes axis formation in the leg^{14,15}. Here we show that the combined action of *wg*-expressing cells in the ventral–anterior compartment and *dpp*-expressing cells in the dorsal–anterior compartment activates expression of *Distal-less*, a gene required for proximal–distal axis formation in the limbs. These results demonstrate that sequential interaction between anterior–posterior and dorsal–ventral compartments establishes the proximal–distal axis of the limbs.

Limb development in *Drosophila* requires the activity of the homeobox gene *Distal-less* (*Dll*; refs 16–19). The limbs develop from imaginal discs, specialized groups of epithelial cells which are recruited in the embryo and grow during larval development to organize the pattern of the adult appendages. Genetic studies have shown that *Dll* activity is required in the imaginal discs to promote formation of the limbs^{16–20}. Figure 1 shows the pattern of *Dll* protein expression at three stages of development of the leg disc. In second instar and early third instar discs, *Dll* is expressed in a subset of cells in the centre of the disc epithelium (Fig. 1a). To accommodate growth during third instar, the central region of the disc epithelium adopts a highly folded arrangement. *Dll* is expressed in the central folds of the mature leg disc (Fig. 1b, c). Fate mapping studies have shown that the central folds correspond to the distal leg segments²¹. In the early pupal leg disc it is apparent that the domain of *Dll* expression corresponds to the distal tibia and tarsal segments of the leg (Fig.

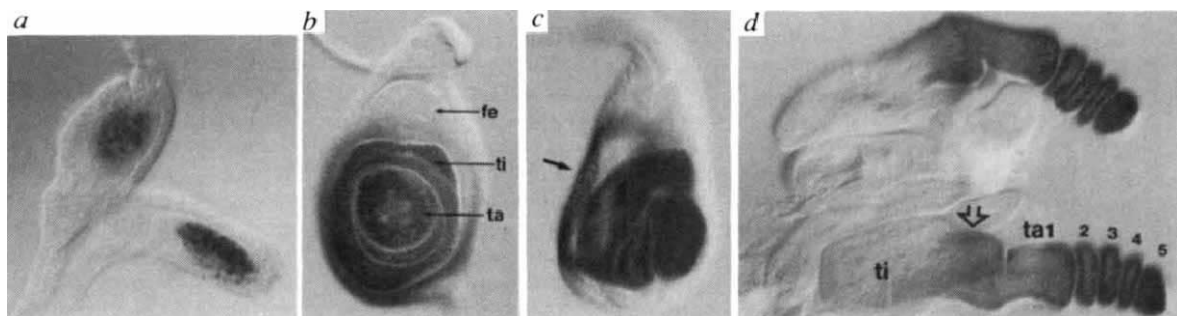
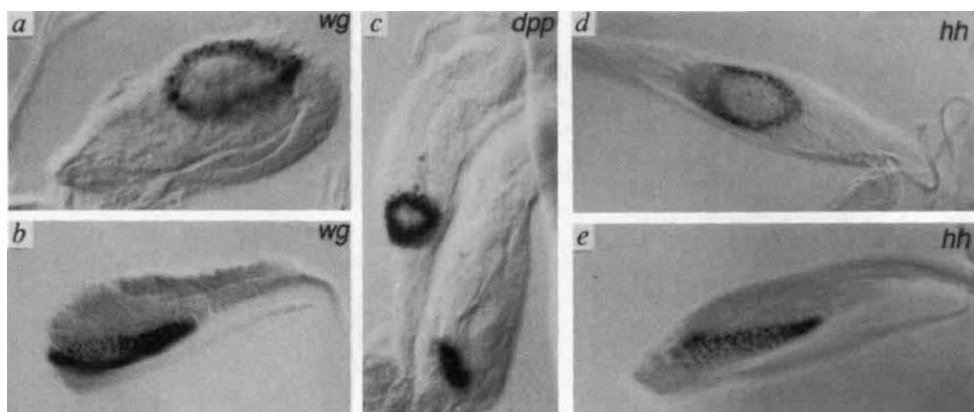


FIG. 1 *Distal-less* expression in the leg imaginal disc. a, *Dll* protein is expressed in a small group of cells in the central region of a late second instar leg disc, where the disc forms a thickened pseudostratified epithelium (visible in the lateral view). b, c, Frontal and lateral views of *Dll* expression in mature third instar leg discs. *Dll* is expressed in a central domain of the disc, corresponding to the presumptive distal segments of the leg (abbreviations: ta, tarsal segments; ti, tibia; fe, femur). In the frontal view (b), the deep folding of the disc epithelium produces a ring-like appearance to the staining. In the lateral view

(c) it is apparent that the central core of the disc constitutes a single contiguous domain of *Dll* expression. A secondary non-contiguous ring of *Dll* expression arises in the proximal femur in mid-third instar discs (arrow). The function of this domain is not known. d, *Dll* expression in everted pupal foreleg discs. The central domain corresponds to the tarsal segments (ta 1–5) and the distal part of the tibia (open arrow). METHODS. *Dll* protein was visualized in imaginal discs using antibody raised in mouse²⁸, as described in ref. 7.

FIG. 2 *hedgehog*, *wingless* and *decapentaplegic* activity are required to activate *Distal-less* expression. *a, b*, Frontal and lateral views of leg discs from third instar larvae carrying the temperature-sensitive mutation *wg*^{tl114}. Embryos were raised at the permissive temperature (17 °C). *Wg* was inactivated by shifting to the restrictive temperature (29 °C) in the second instar. Discs were immunostained to visualize Dll protein. Dll expression is lost from the entire central domain of the disc and the distal structures of the leg fail to develop (compare with wild-type third instar discs in Fig. 1*b, c*). Comparable results are



obtained in the antenna disc (not shown). The secondary ring of Dll expression in the proximal femur is not affected by removing *wg* activity. *c*, Leg discs from *dpp* mutant larvae. *dpp*^{discV} mutants provide *dpp* function in the embryo but lack imaginal disc enhancers, and so do not express *dpp* in the developing leg disc^{29,30}. The entire central domain of Dll expression in the leg is missing from the *dpp*^V discs, but the proximal ring in the femur remains. This phenotype is stronger than that shown for *wg* or *hh* because *dpp* activity is missing throughout disc development. Earlier removal of *wg* produces an equally strong phenotype (not shown). *d, e*, Leg discs from third instar larvae carrying

the temperature-sensitive mutation *hh*^{ts2} shifted to the restrictive temperature in second instar. Dll expression is lost from the central domain and distal structures are missing, as for *wg*.

METHODS. Use of the temperature-sensitive alleles *hh*^{ts2} and *wg*^{tl114} in imaginal discs has been described^{8,12}. *dpp*^{discV} mutant larvae were heterozygous for *ln(2L)dpp*^{d12} and *Df(2L)dpp*^{d14} (ref. 29). Mutant larvae were identified by the absence of the dominant larval marker *Tubby* carried on the TM6B balancer (*hh*) or on the compound balancer SM6A:TM6B (*wg* and *dpp*).

1*d*). Mutations that reduce *Dll* gene activity produce adult flies in which the distal segments of the leg fail to develop. The extent to which distal structures are deleted depends on the severity of the *Dll* mutant allele, suggesting that *Dll* functions in the formation of the proximal-distal axis of the limbs¹⁹. Complete removal of Dll activity from the distal segments results in loss of the entire limb²⁰.

Localized expression of Dll in the central domain of the disc requires the activity of the secreted signalling molecules encoded by the *hh*, *wg* and *dpp* genes (Fig. 2). *wg* is expressed in a wedge-shaped sector in the ventral region of the discs^{10,12}, overlapping the central domain of Dll expression (Fig. 3). Removing *wg* activity during the early third larval instar completely abolishes Dll expression in the central domain of the leg disc causing loss of the distal structures (Fig. 2*a, b*; see also refs 10–12). *dpp* is expressed in a band of anterior cells adjacent to the anterior-posterior (A-P) compartment boundary of the discs^{22,23}, where it overlaps the central domain of Dll expression (Fig. 3). Removing *dpp* activity using disc-specific regulatory mutants leads to loss of Dll expression in the central domain of the leg disc (Fig. 2*c*), consistent with the loss of the appendages in these mutants²⁴. *hh* expression in the posterior compartment of the discs is required for the expression of both *wg* and *dpp* in cells adjacent to the A-P compartment boundary^{8,25}. Removing *hh* activity using a temperature-sensitive mutation also removes Dll expression from the central domain and leads to loss of distal leg structures (Fig. 2*d, e*), presumably by removing *wg* and *dpp* expression. These findings suggest that the combined activity of *wg* and *dpp* is required to activate Dll expression in the leg disc. *wg* and *dpp* have also been shown to direct *aristaless* expression in the leg¹⁵.

The requirement for *hh* activity in leg development appears to be mediated through *wg* and *dpp*. Under normal conditions, *wg* and *dpp* are expressed in cells near the A-P compartment boundary of the leg and antenna discs (illustrated in the diagrams at the bottom of Fig. 3). However, the entire ventral-anterior quadrant of the disc is competent to express *wg* when provided with the *hh* signal⁸. Similarly, the entire dorsal-anterior quadrant is competent to express *dpp*. Under these conditions the interface between cells expressing *wg* and *dpp* expands from a localized region in the centre of the disc to fill the entire anterior compartment (Fig. 3 and ref. 8). As a consequence, the domain

of Dll expression is expanded into a broad stripe of cells straddling the interface between the dorsal-anterior and ventral-anterior quadrants, where the *wg*- and *dpp*-expressing regions meet (Fig. 3*c, d* and *g, h*). These findings support the proposal that the combined activity of *wg* and *dpp* directs Dll expression in the central region of the leg disc.

To test this proposal more directly we examined the consequences of misexpressing *wg* and *dpp* in the discs (Fig. 4). Spatially inappropriate expression of *dpp* in most cells of the leg and antenna discs causes ectopic expression of Dll in the ventral region of the disc, where *wg* is normally expressed (arrows in Fig. 4*a, b*). Localized misexpression of *dpp* in clones of cells can induce bifurcations in the ventral region of the leg (Fig. 4*d, e*). Although the bifurcations are distally incomplete, these results suggest that formation of a secondary proximal-distal axis can be elicited by misexpressing *dpp* in the *wg* domain. Similarly, clones of *wg* expressing cells on the dorsal side of the disc can promote formation of a secondary proximal-distal axis, which is associated with ectopic expression of Dll in the leg disc (Fig. 4*c*). Bifurcations occur only when the clone is close to the A-P compartment boundary, where *dpp* is expressed^{14,15}.

Our findings show that formation of the proximal-distal axis of the leg is initiated by the interaction of distinctly specified cells in adjacent compartments, in agreement with the boundary model²⁶. The activity of the posterior compartment signal *hh* is required to direct expression of *wg* in the ventral region and of *dpp* in the dorsal region of the anterior compartment. Misexpression of *hh* revealed that the anterior compartment is divided into a dorsal quadrant competent to express *dpp* and a ventral quadrant competent to express *wg*⁸. Although it is not clear how these quadrants are established, they may correspond to a proposed subdivision of the anterior compartment of the leg into dorsal and ventral compartments²⁷. Thus interaction between cells in the anterior and posterior compartments establishes the conditions necessary for a subsequent interaction between *dpp*-expressing dorsal cells and *wg*-expressing ventral cells which, in turn, leads to activation of Dll expression in the centre of the disc. Localized expression of the homeodomain protein Dll is required to specify distal cell fates in the limb primordia^{18–20}. Identification of target genes through which Dll acts may help to elucidate the mechanisms by which growth and pattern are controlled to generate the proximal-distal axis of the limbs.

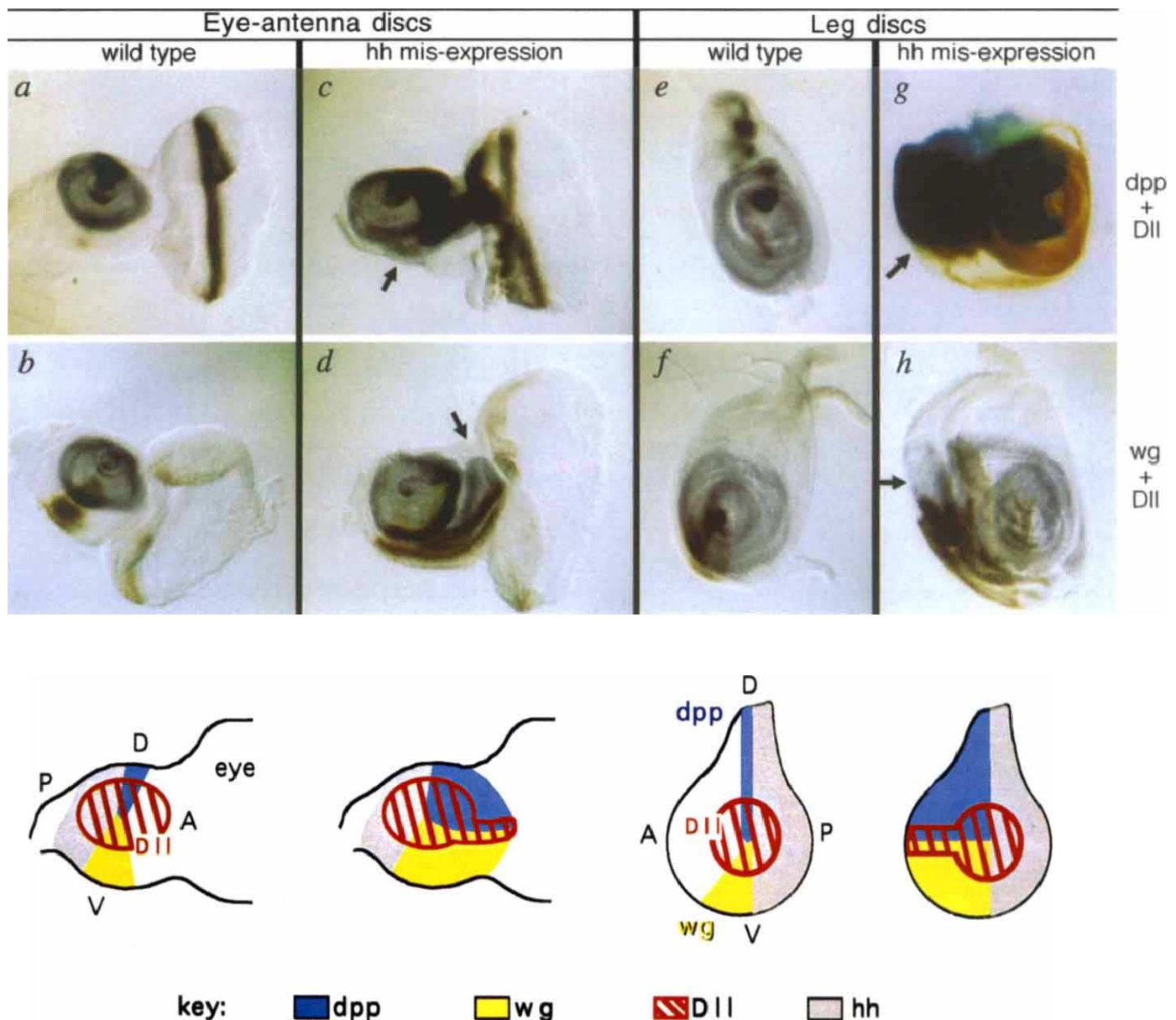
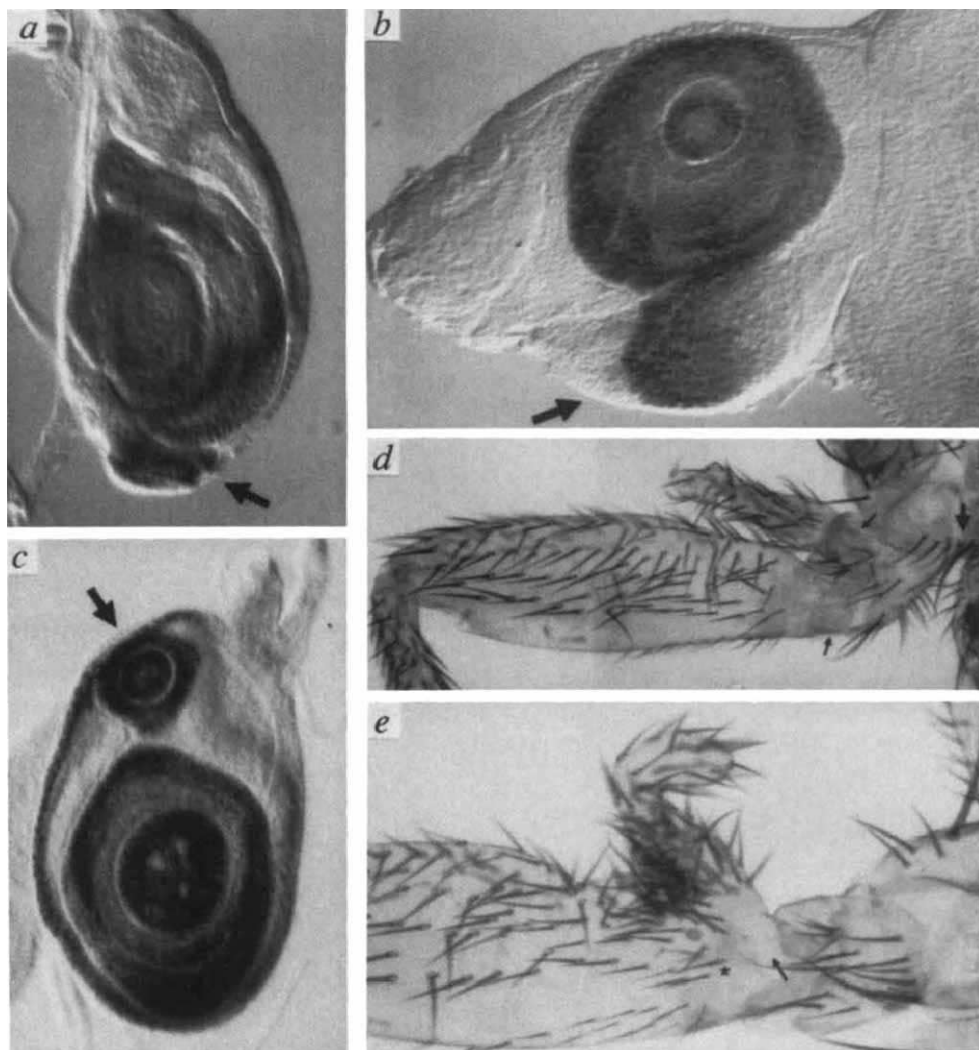


FIG. 3 *hedgehog*-dependent reorganization of the proximal-distal axis involves misexpression of both *wingless* and *dpp*. Double labelling shows the relationship between the spatial domains of *wg*, *dpp* and *Dll* in the imaginal discs. The relationships are summarized in the diagrams at the bottom. *hh* expression in the posterior compartment is depicted in grey. *dpp* is expressed dorsally (blue) and *wg* ventrally (yellow) in the anterior compartment. The central domain of *Dll* expression is depicted by red stripes. Antenna discs are shown in *a-d*; leg discs are shown in *e-h*: wild-type antenna discs are shown in *a, b* and leg discs in *e, f*; discs where *hh* was ectopically expressed are shown in *c, d, g, h*. *Dll*-expressing cells are labelled in grey in all panels except *g* (brown). The top row shows double labelling of *Dll* and *dpp* (*a, c, e, g*). *dpp*-expressing cells are labelled in brown (*a, c, e*; blue in *g*). The middle row shows double labelling of *Dll* and *wg*. *wg-lacZ*-expressing cells are labelled in brown (*b, d, f, h*). Discs are oriented with dorsal to the top. A, anterior; P, posterior; D, dorsal; V, ventral; eye, eye portion of eye-antenna disc. *a, e*, *dpp* is expressed in a stripe of cells that runs along the anterior side of the A-P compartment boundary. The stripe overlaps the central core of *Dll* expression in antenna and leg, and extends along the entire proximal-distal axis. In the antenna (*a*) the stripe appears as a wedge-shaped sector. In the leg (*e*) the stripe appears as an irregular line owing to the extensive folding of the disc epithelium. *dpp* is expressed more strongly on the dorsal side of the disc than on the ventral side in the third instar, but is detectable on both sides. *dpp* is also expressed in the morphogenetic furrow in the eye disc²³. *b, f*, *wg* is expressed in a wedge-shaped sector on the ventral side of the leg and antenna discs

which extends along the entire proximal-distal axis. The sector overlaps the central domain of *Dll* expression. *c, d, g, h*, *Hh* protein was misexpressed using the flip-out technique (ref. 8). In the antenna disc ectopic expression of *Dll* occurs in conjunction with expansion of the domain of *dpp* expression in the dorsal-anterior quadrant (*c*) and of *wg* expression in the ventral-anterior quadrant (*d*) (toward the eye portion of the disc, arrows *c, d*). Note that the domain of ectopic *Dll* expression straddles the interface between *dpp*-expressing dorsal cells and *wg*-expressing ventral cells (as indicated in the diagram at bottom). In both cases *Dll* expression extends some distance into the opposite quadrant (arrows), suggesting that the combined action of *wg* and *dpp* activates *Dll* expression. Identical results are obtained in the leg (*g, h*). *Dll* expression expands along the interface between *wg* and *dpp*-expressing regions (to the left). The ectopic domain of *Dll* overlaps the interface between the dorsal and ventral quadrants (arrows). The spatial relationships and the effects of misexpressing *hh* are identical in leg and antenna, but are easier to visualize in the antenna because the disc is less extensively folded. Note that in *g*, *Dll* is labelled in brown and *dpp* in blue.

METHODS. Larvae heterozygous for *tub > y⁺ > hh*, *HSFLP* and either a *dpp-lacZ* transgene³⁰ or a *wg-lacZ* enhancer detector (*wg^{ro727}* from U. Gaul) were induced to express *hh* by a 1-hour heat shock at 37 °C during third instar⁸. Discs were labelled with mouse antibody to *Dll* and rabbit anti- β -Gal, except in *g*, where X-gal staining was used to visualize *dpp*-expressing cells.

FIG. 4 Ectopic expression of *dpp* or *wg* activates *Dll* expression and reorganizes pattern in the leg and antenna discs. *a, b*, Leg and antenna discs from larvae carrying an *Act5C>y⁺>dpp* transgene. Larvae were exposed to a strong heat shock to induce FLP-induced excision of the *y⁺* cassette, causing *dpp* expression in most cells. *a*, Leg disc showing ectopic expression of *Dll* associated with a localized overgrowth of leg tissue in the *wg*-expressing region on the ventral side of the disc (arrow). *b*, Antenna disc showing ectopic expression of *Dll* ventrally in the *wg*-expressing region (arrow). Some expansion of the ventral region of the disc is evident. Morphological reorganization of the disc can be considerably stronger than in the example shown. Discs are oriented dorsal up. *c*, Ectopic expression of *Dll* in a dorsally located leg bifurcation caused by a clone of *wg*-expressing cells (arrow). Because bifurcation-inducing clones are always located dorsally near the A-P compartment boundary^{14,15}, we infer that the *wg*-expressing clone must lie near the source of the *dpp* signal to cause ectopic expression of *Dll*. The position of the clone was not determined directly. *d*, Bifurcation of a third leg caused by a clone of *dpp*-expressing cells. The cells in the clone are genetically marked with yellow. The clone includes the ventral-most bristle of the coxa segment (large arrow). X-gal staining of adult legs from flies carrying the *wg¹⁰⁷²⁷* transgene shows that this bristle is located in the *wg*-expressing domain (not shown). The clone causes a bifurcation in the trochanter segment, which is distorted. Two separate trochanter-femur joints can be seen (small arrows), as well as a duplication of bristle pattern with the trochanter. An incomplete femur is formed in the bifurcation. The femur and trochanter segments of the bifurcation consist of both *dpp*-expressing and wild-type cells, indicating that the *dpp*-expressing cells can reprogramme the developmental fates of their neighbours. The clone of genetically marked *dpp*-expressing cells extends to the tip of the bifurcation (not visible in this plane of focus). *e*, Bifurcation of the femur induced by a clone of *dpp*-expressing cells. The bifurcation originates in the ventral region of the femur (arrow), next to the ventral-most row of macrochaete in the anterior compartment (asterisk). The leg and bifurcation are twisted so that the bifurcation points toward the top of the figure. Clones of *dpp*-expressing cells were generated in flies carrying two copies of the *Act5C>y⁺>dpp* trans-



gene on chromosome 2. Flies carrying two copies of the transgene produced bifurcations in ~5% of legs carrying clones. The proportion of clones producing a bifurcation was lower in flies carrying one copy of the transgene. Also, the average size of the bifurcations was smaller in flies carrying one copy of the transgene. The dose dependence in frequency and extent of bifurcation suggests that the functional level of *dpp* activity being generated by the *Act5C>y⁺>dpp* construct is lower than the endogenous level. It is possible that a more active construct might generate more complete bifurcations. METHODS. An *Act5C>y⁺>dpp* transgene on chromosome 2 was used to generate clones of cells expressing *dpp* (details available on request). *dpp* expression was induced in most cells after a strong heat shock as described for *wg* and *hh*^{8,15}. Isolated clones of *wg*-expressing cells were induced by brief heat-shock treatment as described^{13,14}.

Note added in proof: It has come to our attention that Held *et al.* have demonstrated genetic interaction between *wg*, *dpp* and *Dll* (*Wilhelm Roux Arch. dev. Biol.* **203**, 310; 1994). Panganiban *et al.* recently described the pattern of *Dll* expression in butterfly legs (*Curr. Biol.* **4**, 671; 1994). □

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Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene

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LEUKOTRIENES constitute a class of potent biological mediators of inflammation and anaphylaxis (for reviews see refs 1 and 2). Their biosynthesis derives from 5-lipoxygenase-catalysed oxygenation of arachidonic acid in granulocytes, macrophages and mast cells. To examine the physiological importance of leukotrienes, we have disrupted the 5-lipoxygenase gene by homologous recombination in embryonic stem cells. 5-Lipoxygenase-deficient ($5\text{LX}^{-/-}$) mice develop normally and are healthy. They show a selective opposition to certain inflammatory insults. Although there is no difference in their reaction to endotoxin shock, the $5\text{LX}^{-/-}$ animals resist the lethal effects of shock induced by platelet-activating factor. Reaction to ear inflammation induced by phorbol ester is normal, whereas inflammation induced by arachidonic acid is markedly reduced. Contrasts were also found in two models of leukocyte chemotaxis *in vivo*. The phenotype of $5\text{LX}^{-/-}$ mice under injurious insult identifies the role for leukotrienes in the pathophysiology of select inflammatory states.

The 5-lipoxygenase gene was disrupted in exon 6 by insertion of a *neo*^r cassette (Fig. 1a). Breeding of F_1 mice (C57Bl/6 $\times$ 129 Sv) heterozygous for the mutation ($5\text{LX}^{+/-}$) resulted in litters of the expected genotype ratio (Fig. 1b). Analysis of macrophages and bone-marrow-derived mast cells from $5\text{LX}^{-/-}$ mice revealed no residual 5-lipoxygenase messenger RNA, protein or ability to synthesize leukotrienes (Fig. 2a–d), indicating a null allele. However, cyclooxygenase-dependent prostaglandin production remained intact (Fig. 2d). Altered protein generated from truncated or exon-skipped transcripts, although not detected, would be non-functional on the basis of *in vitro* mutagenesis studies and the fact that frameshifting would destroy the encoded iron-atom ligands^{3,5}.

$5\text{LX}^{-/-}$ mice show no apparent abnormalities up to ten months of age under normal physiological conditions, except that the spleen is usually smaller (63.1 ± 3.2 mg (mean $\pm$ s.e.m.; range, 46–75.3) versus 79.1 ± 6.2 mg (54.4–115.1) for $5\text{LX}^{+/+}$ 8-week-old mice, $n=11$; $P<0.05$, Student's *t*-test). Analysis of cell-surface markers CD3, CD4 and B220 on splenocytes from 4-week- and 8-week-old mice, as well as histological examination, failed to demonstrate changes in the $5\text{LX}^{-/-}$ mice (data not shown). There was no change in total blood cell populations or differential cell counts, and analysis of the bone marrow revealed no evidence of abnormal precursor cells of any lineage between littermates. Leukotrienes have previously been linked to myelopoiesis and colony-stimulating factor-induced myeloid colony formation^{6,7}. 5-Lipoxygenase products therefore do not seem to be necessary for normal development, nor do they play

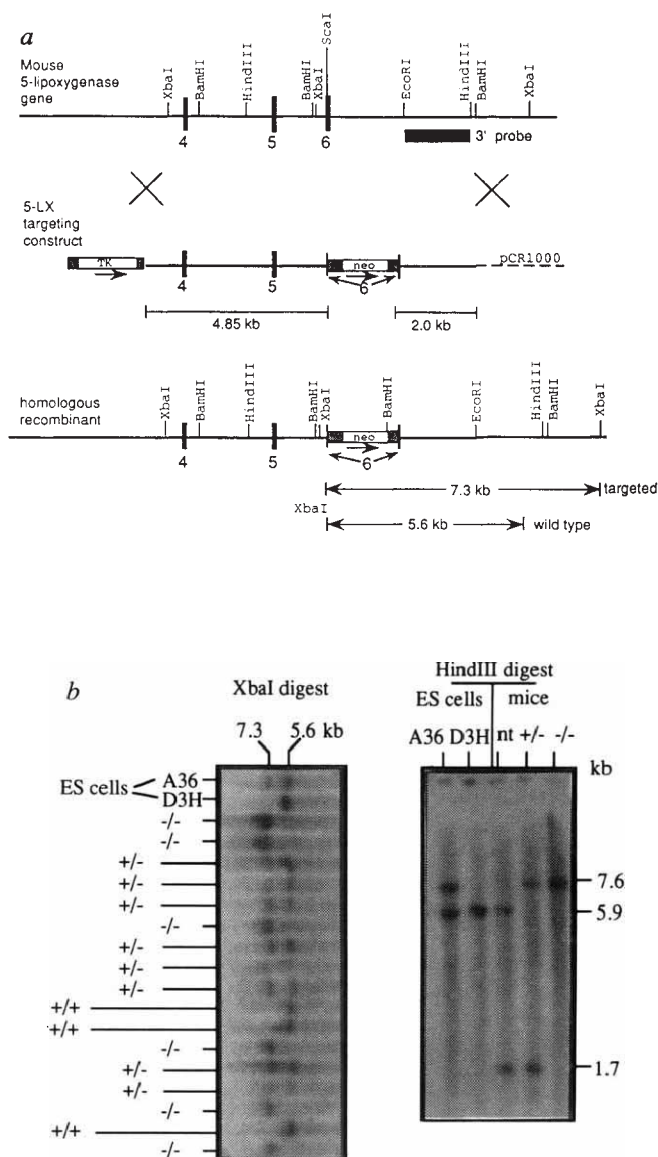


FIG. 1 Targeted inactivation of the mouse 5-lipoxygenase gene. **a**, Partial restriction map of the wild-type allele, the replacement-type targeting vector and the predicted homologous recombinant allele. Exons are indicated by thick vertical bars. **b**, Southern blot analysis of genomic DNA from the parental embryonic stem (ES) cell line D3H (a subline of D3 derived from 129 Sv strain²¹), the targeted ES cell line A36 and offspring from chimaeric backcross and heterozygous interbred mice. A *HindIII* polymorphism became evident upon backcrossing chimaeric males with C57Bl/6 females. nt, non-transmitter. The 7.3-kb *XbaI* fragments and the 7.6-kb *HindIII* fragments were indicative of targeted events.

METHODS. The targeting vector was constructed in the pCR1000 vector (Invitrogen) from a 6.85-kb *SacI*–*EcoRI* 5-lipoxygenase genomic fragment isolated by screening a mouse 129 Sv genomic λ -phage library with a human 5-lipoxygenase cDNA²². A 1.7-kb PGK1neo cassette was inserted into the unique *SalI* site in exon 6 and an *XhoI*–*SalI* thymidine kinase gene for negative selection was placed in an *XhoI* generated site in the vector at the 5' end. D3H-ES cells were electroporated with 25 μg *XhoI*-linearized targeting vector and selected in medium containing G418 (300 $\mu\text{g ml}^{-1}$) and gancyclovir (2 μM). 672 doubly-resistant ES clones were screened by Southern blot analysis as described²³ with a 1.7-kb 3' probe. Further analysis with 5' external and *neo* probes revealed no rearrangements or extra insertional events in seven clones (not shown). Targeted ES cells from cell line A36 were injected into the blastocoel cavity of 3.5-day blastocysts obtained from naturally mated C57Bl/6J mice for subsequent transfer to pseudopregnant ICR recipients. Chimaeric offspring males with high percentage agouti coat colour were mated with C57Bl/6J females.

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strikingly significant biological actions in mice under usual physiological conditions.

Platelet-activating factor (PAF) is an important endogenous mediator of inflammatory, systemic anaphylactic and shock states⁸. In rodents, PAF does not cause platelet aggregation, so the mortality in this model is not attributable to thrombotic complications⁹. Between 62 and 75% of conscious, unanaesthetized 5LX^{+/+} mice die after intravenous (i.v.) injection of two different doses of PAF (140 and 175 $\mu\text{g kg}^{-1}$; Fig. 3a); the animals become listless and usually die within 15–25 min. In contrast, 5LX^{-/-} mice display resistance to the lethal effects of PAF (Fig. 3a). They exhibit the same behaviour for the first 20 min but recover quickly. We did not detect signs of bronchoconstriction in mechanically ventilated, anaesthetized 5LX^{+/+} and 5LX^{-/-} mice, but it was observed after methacholine challenge (data not shown). PAF is known to cause release of peptidyl leukotrienes from perfused rat lungs with subsequent pulmonary vasoconstriction and oedema¹⁰. We made blood pressure recordings via the femoral artery in separate experiments in conscious, unrestrained mice before and after PAF

administration. Mean arterial pressure (MAP) and heart rate were not different between the two groups of mice. MAP changed from 97 ± 5 mm Hg (mean $\pm$ s.e.m.; $n = 5$) to 30–40 mm Hg within 5 min after PAF, remained low for 5–10 min and gradually returned to baseline levels over the course of 3 h. Peptidyl leukotrienes are potent coronary vessel spasmogens released by PAF action in isolated perfused heart preparations^{11,12}. The interplay of complement activation¹³, cytokine and leukotriene release caused by PAF may provoke enough tissue injury, vascular permeability changes and cardiac alterations to be lethal. Our data establish a role for leukotrienes in this process.

In contrast to PAF-induced shock, leukotrienes do not seem to be important mediators of mortality in endotoxin-induced shock (Fig. 3b). Mice were sensitized with an inflammatory stimulus (carrageenan) and challenged with endotoxin¹⁴. No mice in either group died at the lowest dose (2 μg) and comparable numbers died at the higher doses of 10, 25 and 50 μg (Fig. 3b).

Inflammatory models characterized by prostaglandin and leukotriene-dependent components have been reported^{15,16}. Ear

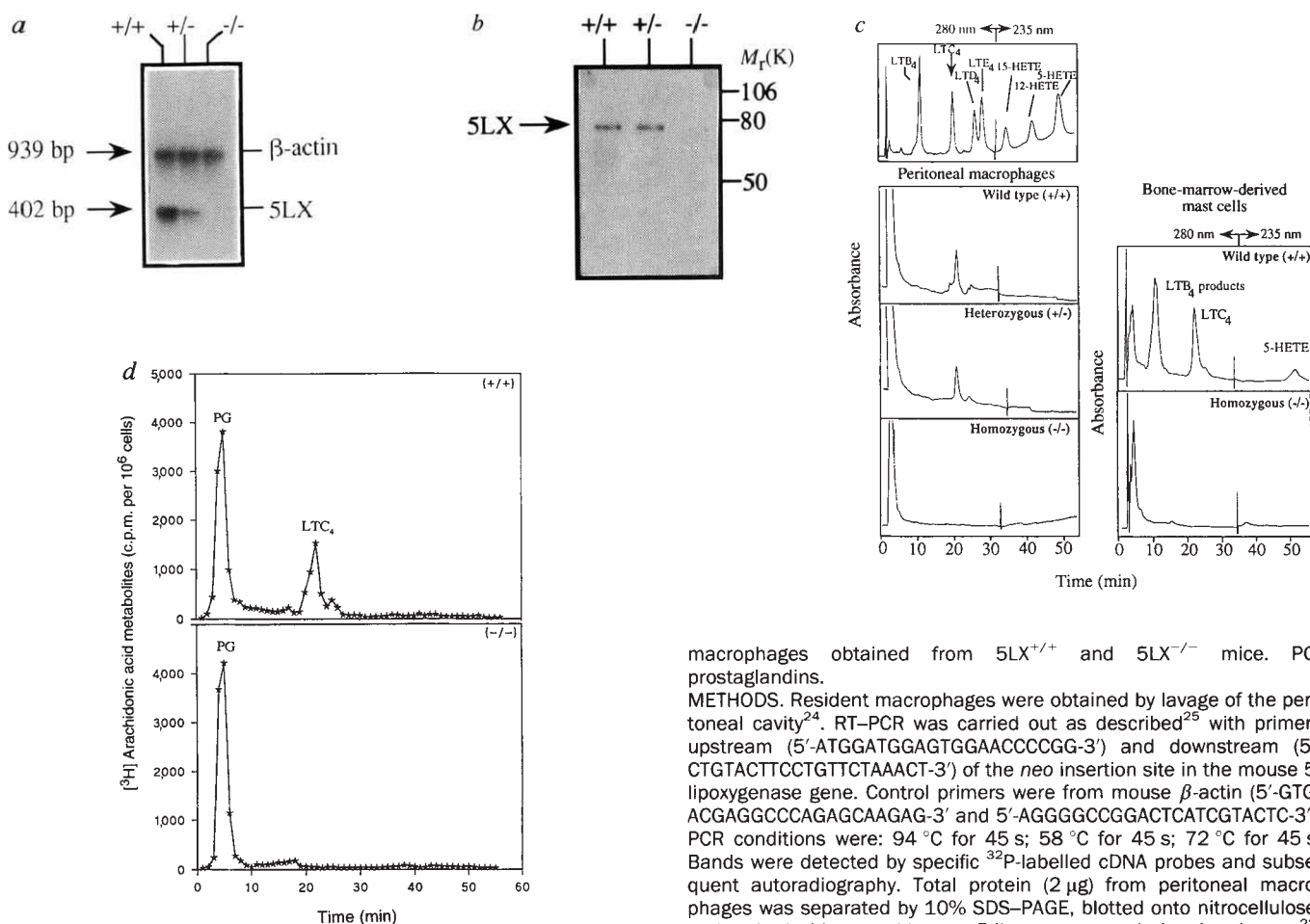


FIG. 2 Absence of 5-lipoxygenase mRNA, protein and leukotriene synthesis in macrophages and bone-marrow-derived mast cells (BMMC) from 5LX^{-/-} mice. **a**, Reverse transcriptase-polymerase chain reaction (RT-PCR) detection of 5-lipoxygenase mRNA. **b**, Western blot detection of 5-lipoxygenase protein. **c**, HPLC chromatograms of products formed from incubations of peritoneal macrophages challenged with zymosan (left) and BMMC challenged with calcium ionophore A23187 (right). The chromatographic profile of several lipoxygenase standards (LTB₄–E₄ and 5-, 12- and 15-HETE) is shown on top left. **d**, Radioactivity profiles of products synthesized from [³H]arachidonic acid prelabelled

macrophages obtained from 5LX^{+/+} and 5LX^{-/-} mice. PG, prostaglandins.

METHODS. Resident macrophages were obtained by lavage of the peritoneal cavity²⁴. RT-PCR was carried out as described²⁵ with primers upstream (5'-ATGGATGGAGTGGAAACCCCGG-3') and downstream (5'-CTGTACTTCTCTTCTAACT-3') of the *neo* insertion site in the mouse 5-lipoxygenase gene. Control primers were from mouse β -actin (5'-GTG-ACGAGGCCAGAGCAAGAG-3' and 5'-AGGGGCCGACTCATCTGACTC-3'). PCR conditions were: 94 °C for 45 s; 58 °C for 45 s; 72 °C for 45 s. Bands were detected by specific ³²P-labelled cDNA probes and subsequent autoradiography. Total protein (2 μg) from peritoneal macrophages was separated by 10% SDS-PAGE, blotted onto nitrocellulose, and probed with an anti-human 5-lipoxygenase polyclonal antiserum²². Macrophages (4×10^6 cells) were incubated with unopsonized zymosan ($200 \mu\text{g ml}^{-1}$) for 2 h, with or without overnight prelabelling with [³H]arachidonic acid. BMMC were prepared and incubated as described²⁶ with 0.5 μM A23187 ionophore for 20 min at 37 °C. Reactions were terminated by the addition of 4 volumes of ethanol. Samples were extracted with ODS-silica columns and products were separated by reverse-phase HPLC (Phenomenex column, 5- μm particle size; 25×0.3 cm) using a solvent system of acetonitrile:methanol:water:trifluoroacetic acid (37.5:25:37.5:0.05) at a flow rate of 0.75 ml min⁻¹. Unreacted substrate was eluted after 60 min by changing the mobile phase to methanol.

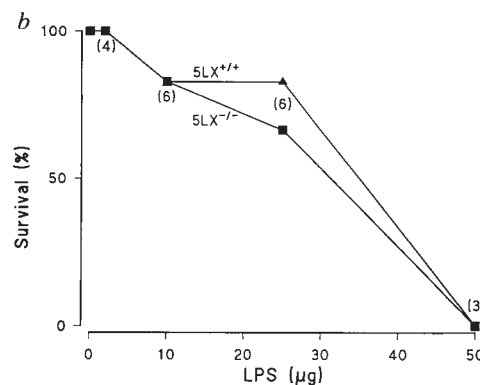
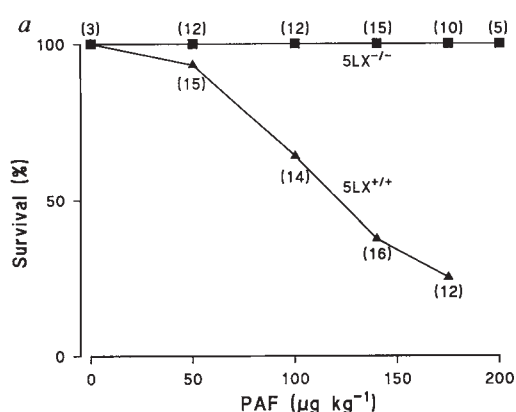


FIG. 3 PAF-induced anaphylactic shock and endotoxin-induced mortality in 5LX^{+/+} and 5LX^{-/-} mice. **a**, Survival curves after PAF injection. **b**, Survival curves after endotoxin injection.

METHODS. Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; Sigma) in chloroform was dried down under argon, resuspended in pyrogen-free normal saline containing 0.25% bovine serum albumin, and injected at the specified doses into the tail vein of conscious male and female mice. Monitoring was carried out for at least 4 h. Pulmonary mechanics in sodium pentobarbital-anaesthetized mice was assessed in other mice in a plethysmographic system according to ref. 27. In separate experiments, mice under pentobarbital anaesthesia (70 mg kg⁻¹) were catheterized with tapered PE-50 tubing

in the femoral artery. The tubing was filled with heparinized saline, fixed at the back of the neck and connected through a rotator cuff to a pressure transducer (P23ID; Gould, Ohio). The signal was transmitted to a polygraph (RS3800; Gould) and blood pressure analyser (Micro-Med). At least 6 h after surgery, blood pressure and heart rate were recorded in conscious, unrestrained mice in a quiet environment before and after i.v. PAF administration²⁸. Male mice (8 weeks old) were pre-treated with 5 mg i.p. carrageenan (Sigma) in 0.5 ml pyrogen-free saline i.p. 24 h before injection (i.v.) of different doses of *E. coli* J5 lipopolysaccharide (mutant of *E. coli* 0111:B4; Calbiochem) and were monitored over 72 h. Numbers of mice used for each point are shown in parentheses.

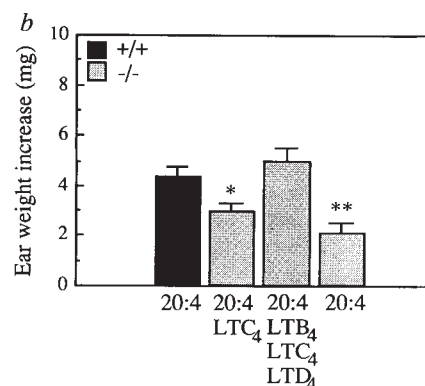
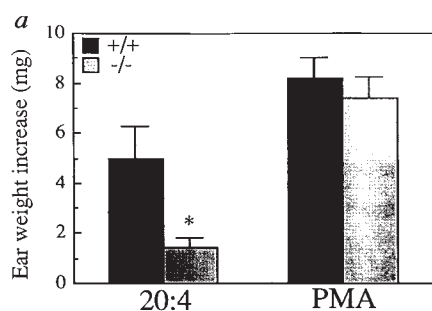
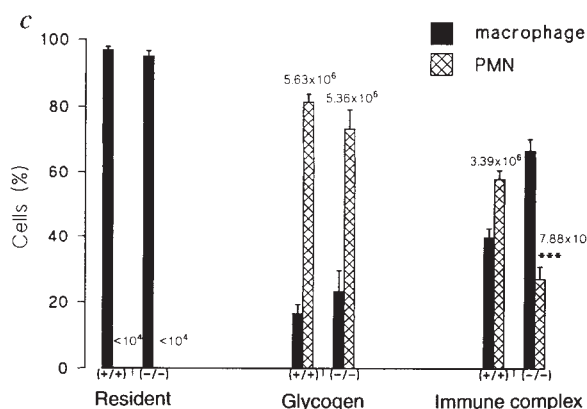


FIG. 4 Induction and evaluation of the inflammatory response in 5LX^{+/+} and 5LX^{-/-} mice. **a**, Ear oedema after arachidonic acid (20:4) or phorbol ester (PMA) treatment; **b**, Effect of exogenous leukotrienes on 20:4-induced ear oedema in 5LX^{-/-} mice; **c**, Recruitment of polymorphonuclear leukocytes (PMN) by glycogen and immune complex challenge. 'Resident' indicates cells lavaged with no prior treatment; glycogen, cells lavaged after 5 h glycogen challenge; immune complex, cells lavaged after 6 h immune complex challenge. Differentials for PMN and macrophages are shown. Lymphocytes and mast cells accounted for less than 5–10% of the total cells. The number of PMN isolated is shown above each cross-hatched column and statistically significant differences were observed between 5LX^{+/+} and 5LX^{-/-} mice challenged with immune complexes (***) $P < 0.0001$; $n = 19$ for 5LX^{+/+} and $n = 15$ for 5LX^{-/-} mice).

METHODS. The inner surface of the left ears from 8-week-old male (a) or female (b) mice was treated with vehicle (acetone) and right ears with arachidonic acid (20:4; 2 mg in 20 µl) or phorbol myristyl acetate (PMA; 2 µg in 20 µl). Exogenous leukotrienes (50 ng each) resuspended in normal saline or saline alone were injected i.d. immediately before 20:4 application. Animals were killed after 1 h (20:4; $n = 6-8$) or 4 h (PMA; $n = 5$) and ear biopsies (6 mm diameter) were weighed immediately. * $P < 0.05$, ** $P < 0.005$. Female mice (6–9 weeks) were injected intraperitoneally with 1% sterile glycogen (2.0 ml) to elicit PMN recruitment. Alternatively, an immune complex peritonitis model¹⁹ was initiated by i.v. injection of ovalbumin (20 mg kg⁻¹) followed by i.p. rabbit anti-ovalbumin IgG (300 µg in normal saline; Cappel) or saline control.



The peritoneal cavity was lavaged at set times and cells were counted and prepared for differential analysis. Data are presented as mean $\pm$ s.e.m. analysed by Student's *t*-test.

inflammation was induced in $5LX^{+/+}$ and $5LX^{-/-}$ mice by topical application of phorbol myristyl acetate or arachidonic acid (20:4). Phorbol ester induced a 95% increase in ear weight at 4 h in control mice when compared to vehicle-treated ears (Fig. 4a). The increase was not significantly different in $5LX^{-/-}$ mice. The phorbol ester model of inflammation has been proposed to be mediated primarily by prostaglandins¹⁵. Arachidonic acid induced a 59% increase in ear weight in $5LX^{+/+}$ mice at 60 min (Fig. 4a). Histological analysis demonstrated evidence of oedema, extensive disruption of the connective tissue layers of the dermis and dilated, congested vessels. Inflammation was significantly diminished in $5LX^{-/-}$ mice (Fig. 4a, b). Administration of leukotrienes to $5LX^{-/-}$ mice enabled the inflammatory phenotype in this model (Fig. 4b) to be 'rescued'; inflammation is rapid in onset and maximal swelling is reached between 30–60 min. Previous studies with lipoxygenase inhibitors have suggested roles for, or against, lipoxygenase products in this inflammation^{16,17}. Our results show that $5LX^{-/-}$ mice can be used to discriminate between models of inflammation.

Neutrophil recruitment into the peritoneal cavity by a non-specific inflammatory challenge (glycogen¹⁸) and by immune-complex formation (reverse passive Arthus reaction¹⁹) was evaluated to examine the role of 5-lipoxygenase products. Resident cells consist primarily of macrophages and less than 1% polymorphonuclear leukocytes (Fig. 4c). Glycogen elicited a large influx of polymorphonuclear leukocytes in both $5LX^{+/+}$ and $5LX^{-/-}$ mice, the difference not being statistically significant (Fig. 4c). On the other hand, induction of immune complex peritonitis elicited a substantially reduced chemotactic infiltration by polymorphonuclear leukocytes in $5LX^{-/-}$ mice (Fig. 4c). Immune-complex-induced injury is a key component of the pathogenesis of antibody-mediated glomerulonephritis, arthritis and other diseases. Our data, combined with previous findings with mast-cell-deficient mice^{19,20}, indicate that the chemotactic leukotrienes released by mast cells are important in neutrophil recruitment during the early acute inflammatory insult.

Our results point to a selective involvement of 5-lipoxygenase products in defined inflammatory states. The targeted disruption of a gene involved in the arachidonic acid cascade illustrates the strength of this technique to discriminate between inflammatory models and also sets the stage to explore the relationship between leukotrienes and other mediators implicated in diverse immune responses. □

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Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1

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INSULIN receptor substrate-1 (IRS-1) is the major substrate of insulin receptor and IGF-1 receptor tyrosine kinases; it has an apparent relative molecular mass of 160–190,000 (M_r , 160–190K) on SDS polyacrylamide gel^{1–3}. Tyrosine-phosphorylated IRS-1 binds the 85K subunit of phosphatidylinositol 3-kinase^{4,5} which may be involved in the translocation of glucose transporters^{6,7} and the abundant src homology protein (ASH)/Grb2^{8,9} which may be involved in activation of p21^{ras} and MAP kinase cascade¹⁰. IRS-1 also has binding sites for Syp¹¹ and Nck¹² and other src homology 2 (SH2) signalling molecules¹⁰. To clarify the physiological roles of IRS-1 *in vivo*, we made mice with a targeted disruption of the *IRS-1* gene locus. Mice homozygous for targeted disruption of the *IRS-1* gene were born alive but were retarded in embryonal and postnatal growth. They also had resistance to the glucose-lowering effects of insulin, IGF-1 and IGF-2. These data suggest the existence of both IRS-1-dependent and IRS-1-independent pathways for signal transduction of insulin and IGFs.

The role of IRS-1 in signal transduction of insulin is suggested by several lines of evidence. In Chinese hamster ovary cells over-expressing mutant insulin receptors, insulin action appears to be parallel with the tyrosine phosphorylation of IRS-1 rather than

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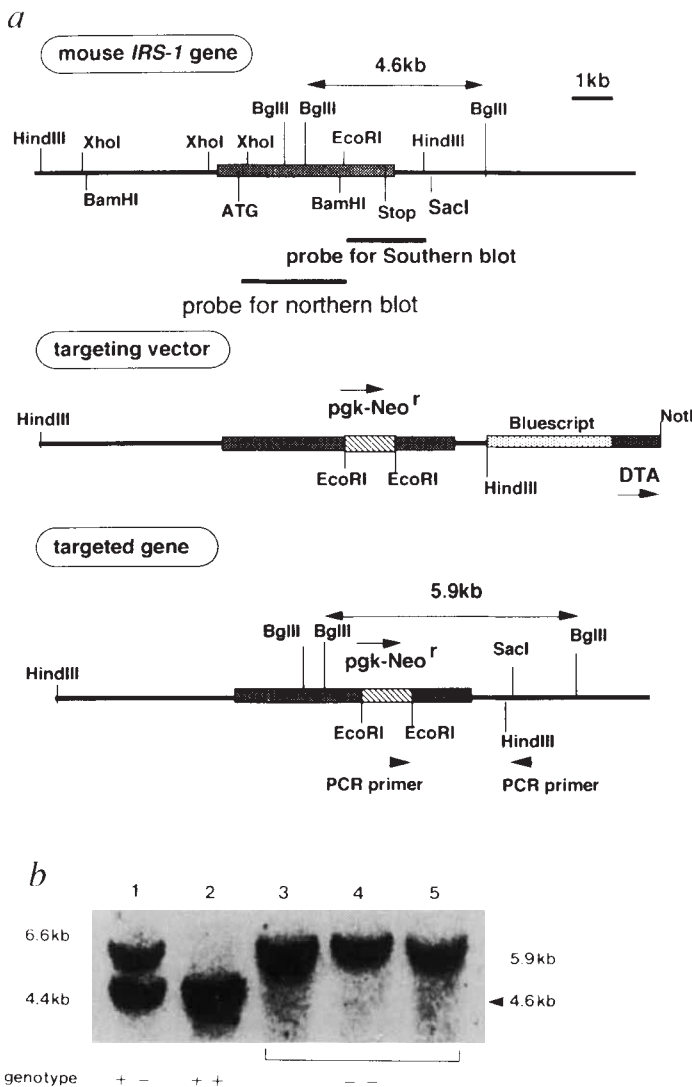


FIG. 1 a, Schematic representation of mouse *IRS-1* gene, targeting vector and targeted allele. The box indicates the first exon of the *IRS-1* gene. With our first targeting vector in which a neomycin-resistant gene (*neo*^r) was placed just 3' to the ATG translation initiation codon, no homologous recombinant clone was obtained. Homologous recombinant clones were obtained with the second vector indicated in the figure. In this vector, *neo*^r with *pgk-1* promoter and without poly(A)⁺ addition signal was inserted into the *EcoRI* site about 2.6 kb 3' to the translation initiation codon of the mouse *IRS-1* gene. Because there are several stop codons in the *pgk-1* gene promoter, this should destabilize the mRNA of *IRS-1*²⁵. The diphtheria toxin A fragment (DTA) gene with MC1 promoter was ligated on the 3' terminus across the vector backbone for negative selection²⁶. Primers for polymerase chain reaction (PCR) used in the selection of homologous recombinants and probes for Southern and northern blot analysis are indicated. **b**, Example of Southern blot analysis of the F2 mice. The genomic DNA extracted from the tail was digested with *BglII* and hybridized with the 1.8 kb *EcoRI*–*HindIII* *IRS-1* gene fragment under high stringency. The wild-type allele gave a 4.6 kb band and the recombinant allele gave a 5.9 kb band. *M*, markers in kb are indicated on the left. Lane 1 indicates heterozygous, lane 2 indicates wild-type and lanes 3 to 5 indicate homozygous mice. The ratio of wild-type, hetero- and homozygous mice at 3 weeks of age was, however, 1:2:0.67 among 230 offspring analysed.

METHODS. The mouse *IRS-1* gene was cloned from BALB/c mouse genomic library using a 400 bp PCR product flanking the ATG translation initiation codon of rat *IRS-1* as a probe. The targeting vector (24 nM) and the embryonic stem cell suspension (1×10^7) were mixed and electroporated at 0.25 V, 960 μ F, using a Biorad Genepulser. Forty-eight hours after electroporation, G418 was added to the medium at the concentration of 150 μ g ml⁻¹. After 5 to 7 days, the G418-resistant clones were isolated and subjected to screening for homologous recombination by PCR. The sense primer was 5'-TTCTATCGCCTTCTTGACG-AGTTC-3' in 3' terminus of *neo*^r, and the antisense primer was 5'-TGCTTTGGTACTGTGAAATCACAG-3' in the intron of *IRS-1* gene. The reaction was started at 95 °C for 5 min, and continued at 72 °C for 7 min, during which time Taq polymerase (2.5 U per tube) was added. Thereafter, 35 cycles of 94 °C (1 min), 62 °C (1 min) and 72 °C (2 min 30 s) were done. Homologous recombinant clones gave 1.8 kb fragment.

that of the receptor itself^{13,15}. Overexpression of *IRS-1* in 32D cells enhances DNA synthesis stimulated by insulin or IGF-1¹⁶. By contrast, several proteins may be involved in *IRS-1*-independent pathways in the signal transduction of insulin. In the blood cell line FDC, a protein called 4PS is tyrosine phosphorylated by insulin and IGF-1 and can bind phosphatidylinositol-3-OH kinase (PI(3)K) and ASH/Grb2¹⁷. A transforming protein Shc is also tyrosine phosphorylated by insulin and IGF-1 and also binds ASH/Grb2^{18,19}. In adipocytes, a 60K protein (pp60) is tyrosine phosphorylated by insulin^{20,21}, and binds PI(3)K²². To understand better the roles of *IRS-1* in normal physiology, we made mice with a targeted disruption of the *IRS-1* gene locus (Fig. 1a).

The male chimaeras that originated from the two embryonic stem cell clones transmitted mutant *IRS-1* allele to their offspring. The heterozygous mice were apparently normal, and gave birth to mice homozygous for the mutant *IRS-1* locus (Fig. 1b). Northern blot analysis was done using liver poly(A)⁺ RNAs with a 2.5 kilobase (kb) *XhoI*–*EcoRI* fragment which is 5' to the insertion site of the *neo*^r as a probe (Fig. 2a). No hybridizing transcript was detected in the homozygous mice. Thus, the insertion of the *neo*^r gene appeared to cause a severe decrease in the expression level of the *IRS-1* gene. Western blot analysis was also done. In immunoprecipitates with polyclonal anti-*IRS-1* antibody (α -*IRS-1*), no tyrosine-phosphorylated band corresponding to *IRS-1* (M_r ~185K) or truncated *IRS-1* was detected in the liver lysate of the homozygous mice (Fig. 2b). In contrast, a distinct band was observed in that of wild-type mice. In

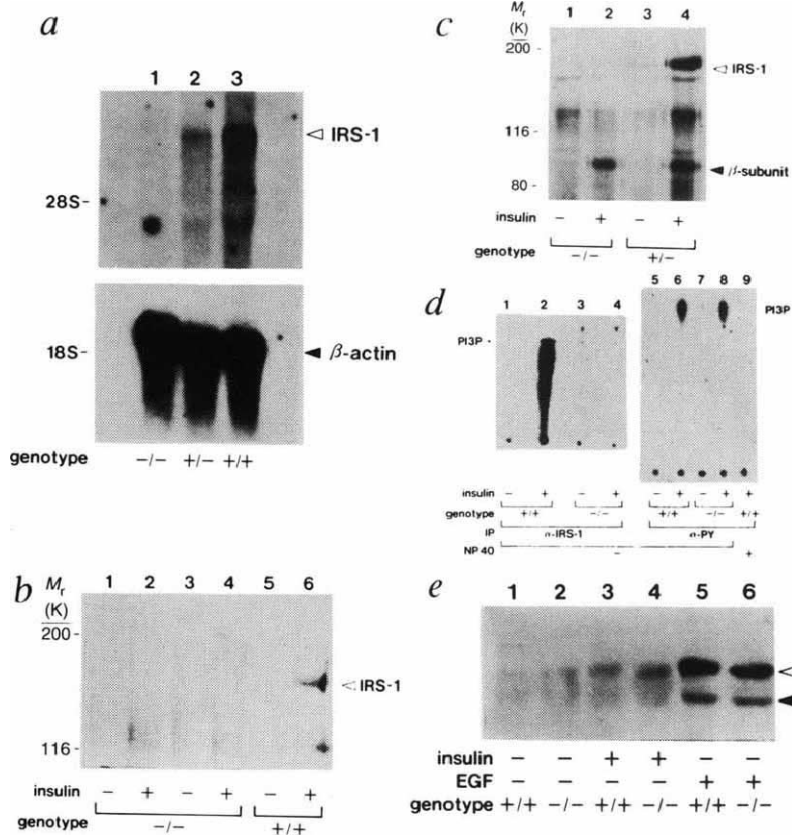
immunoprecipitates with anti-phosphotyrosine antibody (α -PY), *IRS-1* as well as the insulin receptor β -subunit was tyrosine phosphorylated in an insulin-dependent fashion in heterozygous mice (Fig. 2c) and wild-type mice (data not shown). In contrast, there was no insulin-stimulated tyrosine-phosphorylated bands suggestive of truncated *IRS-1* protein in homozygous mutants, whereas the insulin receptor β -subunit was clearly tyrosine phosphorylated. Thus, we were not able to obtain any evidence of expression of the *IRS-1* gene in the homozygous mutant mice.

The PI(3)K activity associated with α -*IRS-1* immunoprecipitates was markedly stimulated in wild-type mice after insulin injection as shown in lanes 1 and 2 of Fig. 2d. Little or no PI(3)K activity was coimmunoprecipitated in α -*IRS-1* immunoprecipitates from the liver of homozygous mutant mice as shown in lanes 3 and 4. In α -PY immunoprecipitates, however, insulin-stimulated activation of PI(3)K was observed (lanes 7 and 8). Shc was normally phosphorylated and MAP kinase activity was only slightly but not significantly reduced in the liver of homozygous mutant mice (Fig. 2e and legend).

Growth of the homozygous mice was retarded (Fig. 3a). At the age of 3, 8 and 15 weeks, the weight was about 30% less than that of normal littermates (Fig. 3a legend); the serum IGF-1, IGF-2 and growth hormone levels were normal in the mutant mice (data not shown). No abnormalities were found in histological examinations of brain, heart, liver, spleen or kidney (data not shown). Both mutant females and males were fertile. The growth retardation of the embryos was apparent at 15.5 embryonic days (Fig. 3b) and the weight of homozygous

FIG. 2 a, Northern blot analysis of the F2 mice. Poly(A)⁺ RNAs from the liver of homozygous mutant (lane 1), heterozygous (lane 2) and wild-type mice (lane 3) were hybridized with either *IRS-1* (upper panel) or the β -actin gene (lower panel). b, Tyrosine phosphorylation of IRS-1 immunoprecipitated from the liver lysate with α -IRS-1 raised against recombinant fusion protein of IRS-1. The immunoprecipitates from the liver lysate of the wild-type (lanes 5 and 6) and homozygous mice (lanes 1 to 4) which were injected with insulin (lanes 2, 4, 6) or were not injected (lanes 1, 3, 5) were blotted with a monoclonal α -PY (PY20). M_r markers (in K) are on the left. c, Phosphotyrosine proteins in the liver of the homozygous mutant mice. Tyrosine phosphorylated proteins were immunoprecipitated with PY20 from the liver lysate of homozygous mutant (lanes 1 and 2) and heterozygous mice (lanes 3 and 4) which were injected with insulin (lanes 2 and 4) or were not injected (lanes 1 and 3) and blotted with the same antibody. d, Activation of PI(3)K. PI(3)K activities associated with either α -IRS-1 (lanes 1 to 4) or α -PY (lanes 5 to 9) in the liver lysates of the homozygous mutant mice injected with insulin (lanes 4 and 8) or without injection (lanes 3 and 7) were compared with those of the wild-type mice injected with insulin (lanes 2 and 6) or without injection (lanes 1 and 5). The PI(3)K activity was inhibited by the presence of 0.5% Nonidet P-40 in the reaction mixture (lane 9). PI3P indicates the position of phosphatidylinositol phosphate standard prepared by phosphorylating phosphatidylinositol with purified PI(3)K. IP indicates antibodies used for immunoprecipitation. e, Tyrosine phosphorylation of Shc. Immunoprecipitates with polyclonal anti-Shc antibody (UBI) from the liver lysates of the homozygous mutant (lanes 2, 4, 6) and wild-type mice (lanes 1, 3, 5) were blotted with α -PY (RC20). The mice were injected with EGF (lanes 5 and 6) or insulin (lanes 3 and 4) or were not injected (lanes 1 and 2). The white arrow indicates 52K Shc and the black arrow indicates 46K Shc. Activity of MAP kinase was stimulated 1.7 ± 0.17 -fold in the homozygous mutant mice and 2.08 ± 0.40 -fold (mean $\pm$ s.e.m.) in the wild-type mice.

METHODS. a, Poly(A)⁺ RNA (3 μ g) was resolved through 0.7% formaldehyde agarose gel and transferred onto nylon membrane. The membrane was hybridized under high stringency with 2.5 kb *XhoI-EcoRI* *IRS-1* gene fragment. The integrity of RNA was confirmed by hybridizing a duplicated membrane with human β -actin gene fragment. b, Mice were anaesthetized and 10 μ g per g body weight of human insulin was injected through the portal vein. After 1 min the liver was removed and homogenized in ice-cold homogenization buffer A (25 mM Tris-HCl, pH 7.5, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 1 mM sodium vanadate, 40 mM *p*-nitrophenylphosphate (PNPP), 1 mM phenylmethylsulphonyl fluoride, 10 mM EDTA and 10 mM EGTA). The homogenate was centrifuged and the supernatants were subjected to immunoprecipitation with 30 μ l of α -IRS-1 (1-6 or B56). For immuno-



precipitation with α -PY, liver lysates were prepared as described in ref. 27 and immunoprecipitated with α -PY (PY20). After washing, the immunoprecipitates were blotted with α -PY. α -IRS-1 1-6 was raised against fusion protein of IRS-1 corresponding to residues 778-1,243, α -IRS-1 B56 was raised against synthetic peptide YIPGATMGTSPLTGDE (residues 489-505). d, The liver homogenates were prepared in homogenization buffer A without PNPP and precleared with wheat-germ agglutinin agarose before immunoprecipitation with α -PY (PY20) or α -IRS-1 (B56), then subjected to PI(3)K assay as described⁹. e, Insulin-stimulated liver lysates were prepared as above. EGF was injected intraperitoneally. The liver lysates were incubated with polyclonal antibodies against a GST-fusion protein of SH2 domain of Shc (UBI) or polyclonal anti-ERK1 antibody²⁸. The immunoprecipitates were subjected to western blotting with alkaline phosphatase conjugated monoclonal α -PY (RC20, Transduction Laboratories). MAP kinase activity was analysed with in-gel phosphorylation assay²⁸.

embryos was about 80% of that of normal and heterozygous embryos at 18.5 embryonic days (Fig. 3b legend).

The blood glucose levels and serum insulin levels before and after oral glucose load were analysed. Although there was no significant difference in levels of the blood glucose among the three genotypes (Fig. 4a, upper panel), the serum insulin level before and after glucose load was significantly higher in the homozygous mutant mice than in wild-type mice and heterozygous mice (Fig. 4a, lower panel). In accord with these data, the drop in the blood glucose levels after injection of human insulin was significantly smaller in homozygous mutant mice than those of heterozygous and wild-type mice (Fig. 4b). Similar results were observed with IGF-2 injection (Fig. 4c) and also with IGF-1 (data not shown). Thus, homozygous mutant mice have resistance to IGF-1 and IGF-2 in addition to insulin. The stimulation of 3-O-methylglucose transport activity of the isolated adipocytes with insulin was significantly impaired in homozygous mice compared with that of the wild-type mice (Fig. 4d).

This study has shown that IRS-1 is required at least in part for the pre- and postnatal growth-promoting effect and glucose-lowering effect of insulin, IGF-1 and IGF-2. The growth retardation of the IRS-1 knockout mice was milder than that of the IGF-1 receptor knockout mice²³, perhaps suggesting the existence of the IRS-1-independent pathways in signal transduction through insulin and IGF-1 receptor tyrosine kinases. In fact, PI(3)K activity appeared to be associated with insulin-stimulated tyrosine-phosphorylated protein(s) other than IRS-1 in homozygous mutant mice (Fig. 2d). Interestingly, we observed a tyrosine-phosphorylated band, with apparent M_r of 190K in insulin-stimulated liver total lysates, which was not recognized with α -IRS-1 in mutant mice (data not shown). Moreover, Shc protein was normally tyrosine phosphorylated in the liver of the homozygous mice (Fig. 2e). MAP kinase activity immunoprecipitated from the liver homogenates was only slightly decreased in homozygous mutant mice. Thus, in the activation of both PI(3)K and Ras by insulin, there appear to be IRS-1-dependent and IRS-1-independent pathways.

FIG. 3 *a*, Postnatal growth retardation of the mice homozygous for the mutation on IRS-1 locus. Body weight was measured from the time of tail cut. The body weight of the wild-type (filled circles), heterozygous (triangles) and the homozygous mutant mice (unfilled circles) was plotted against days after birth. At 3, 8 and 15 weeks of age, the homozygous mice were 5.96 ± 0.38 g, 13.63 ± 0.73 g and 15.84 ± 0.72 g, whereas the wild type were 9.92 ± 0.50 g, 18.08 ± 0.46 g and 25.00 ± 1.49 g, respectively (mean $\pm$ s.e.m.) ($P < 0.01$). *b*, Prenatal growth retardation of mice homozygous for the mutation on IRS-1 locus. Pregnant heterozygous mice were killed at indicated points after mating. The weight of the wild-type (filled circles), heterozygous (triangles) and homozygous mutant embryos (unfilled circles) was plotted against embryonal day. At 18.5 days after mating, the homozygous embryos were 1.07 ± 0.11 g and the wild type were 1.318 ± 0.05 g. ** Indicates $P < 0.01$ and * indicates $P < 0.05$.

METHODS. *a*, Genotype of the F2 mice was determined by PCR. The genomic DNA was extracted from the tails of the mice or liver of the embryo. The sense primers were 5'-TTCTATCGCCTTCTTGACGAGTTC-3' derived from *neo*^r and 5'-GCAGCCCCACCTGCCTCGAAAGGTAGACAC-3' derived from the *IRS-1* gene, and the antisense primer was 5'-CAGCAATGCCTGTCCGCATGTCAGCATAGC-3'. The thermal cycle reaction was the same as that for screening of embryonic stem cell clones. The wild-type allele gave 560 bp, and the recombinant allele gave 360 bp. Genotypes were confirmed by Southern blot analysis. *b*, The embryos were washed with PBS and the weight was recorded. A total of 74 embryos were analysed. The data were analysed with one-way analysis of variance (ANOVA), and multiple comparison methods of Fisher protected least significant difference (P.L.S.D.) were used.

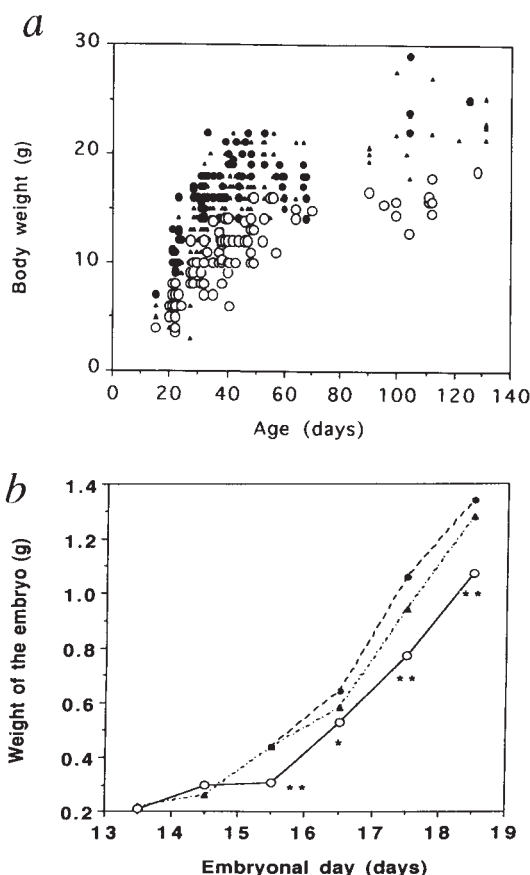
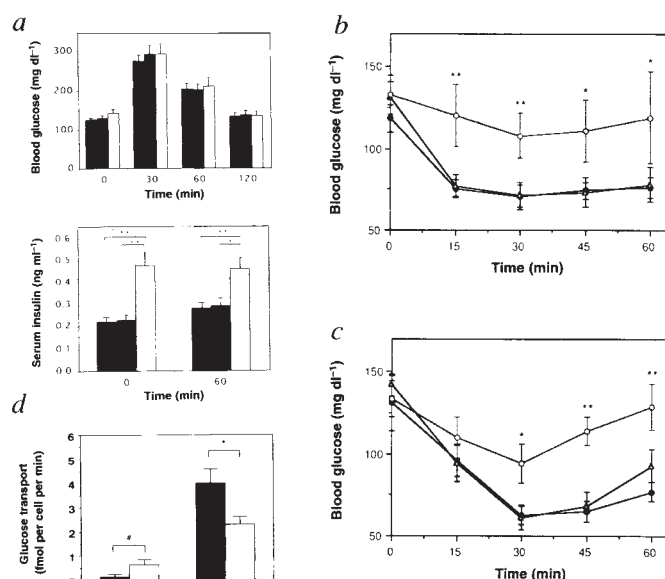


FIG. 4 *a*, Blood glucose and serum insulin after oral glucose load. The wild-type (filled bars), heterozygous (hatched bars) and the homozygous mutant mice (unfilled bars) were loaded with glucose. The blood glucose (upper panel) and serum insulin (lower panel) levels were determined at indicated time points. The bars show mean and standard error of the mean. The serum insulin levels of the homozygous mice before and after glucose load (0.41 ± 0.04 ng ml⁻¹ to 0.46 ± 0.04 ng ml⁻¹) were significantly higher than those of the wild type (0.24 ± 0.02 ng ml⁻¹ to 0.31 ± 0.03 ng ml⁻¹) and heterozygous mice (0.24 ± 0.02 ng ml⁻¹ to 0.37 ± 0.04 ng ml⁻¹) ($P < 0.01$ and $P < 0.05$, respectively). *b*, Insulin tolerance test. *c*, IGF-2 tolerance test. Homozygous (unfilled circles), heterozygous (triangles) and wild-type (filled circles) mice were injected either with human insulin, IGF-1 or IGF-2 into the peritoneal cavity and the blood glucose levels were determined at indicated times. ** Indicates $P < 0.01$ and * Indicates $P < 0.05$. *d*, 3-O-methylglucose transport activity. The filled bars indicate wild-type, and the unfilled bars indicate the homozygous mice. The basal glucose transport of the adipocytes from the homozygous mice (0.64 ± 0.21 fmol per cell per min) was higher than that of the wild type (0.15 ± 0.09 fmol per cell per min) ($P < 0.1$). The insulin stimulated glucose transport of the adipocyte from the homozygous mice (2.31 ± 0.31 fmol per cell per min) was significantly impaired as compared with that of the wild type (4.00 ± 0.57 fmol per cell per min) (mean $\pm$ s.e.m.; $P < 0.05$).

METHODS. *a*, The mice were fasted for 12–15 h before the study, and were then loaded with 1.2 g kg⁻¹ glucose. Blood samples were taken from the orbital sinus. The insulin levels were determined using an insulin RIA kit (Shionogi) with rat insulin as standard. *b*, *c*, Mice of each genotype of about the same age were fed freely before and fasted during the study. Human insulin (0.75 U kg⁻¹) or 1 mg kg⁻¹ of recombinant human IGF-1 (provided by Fujisawa Pharmaceutical) or 0.5 mg kg⁻¹ of recombinant IGF-2 (provided by Daiichi Pharmaceutical) was injected intraperitoneally. Results are presented



as the mean $\pm$ s.e.m.; data were analysed with one-way analysis of variance (ANOVA), and multiple comparison methods of Fisher P.L.S.D. were used, with a significance level of 0.01 or 0.05. *d*, Adipose cells from mouse epididymal fat pads were prepared as described in ref. 29. 3-O-methylglucose transport activity was analysed as described in ref. 30.

Finally, these mice will serve as an animal model for the insulin-resistant state with a known molecular defect, because the phenotype of *IRS-1* knockout mice (mild to moderate post-receptor insulin resistance with normal glucose tolerance) resembles the phenotypes of non-insulin-dependent diabetes mellitus at the prediabetes stage²⁴.

Note added in proof: We have found that activation of PI(3)K in α -PY immunoprecipitates is also present in the muscle in the mutant mice, although somewhat reduced. □

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Alternative pathway of insulin signalling in mice with targeted disruption of the *IRS-1* gene

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THE principal substrate for the insulin and insulin-like growth factor-1 (IGF-1) receptors is the cytoplasmic protein insulin-receptor substrate-1 (IRS-1/pp185)^{1–7}. After tyrosine phosphorylation at several sites, IRS-1 binds to and activates phosphatidylinositol-3'-OH kinase (PI(3)K)^{8–11} and several other proteins containing SH2 (Src-homology 2) domains^{12–14}. To elucidate the role of IRS-1 in insulin/IGF-1 action, we created IRS-1-deficient mice by targeted gene mutation. These mice had no IRS-1 and showed no evidence

of IRS-1 phosphorylation or IRS-1-associated PI(3)K activity. They also had a 50 per cent reduction in intrauterine growth, impaired glucose tolerance, and a decrease in insulin/IGF-1-stimulated glucose uptake *in vivo* and *in vitro*. The residual insulin/IGF-1 action correlated with the appearance of a new tyrosine-phosphorylated protein (IRS-2) which binds to PI(3)K, but is slightly larger than and immunologically distinct from IRS-1. Our results provide evidence for IRS-1-dependent and IRS-1-independent pathways of insulin/IGF-1 signalling and for the existence of an alternative substrate of these receptor kinases.

To create the targeting vector, the mouse *IRS-1* gene containing the 5' flanking region, first exon (which includes the 5' untranslated region, complete coding region, and the first 24 bases of the 3' untranslated region) and a part of the first intron was cloned from a mouse genomic library⁵ and modified by deletion of bases –77 to +179 (removing the start codon and producing a shift in reading frame). In place of this deletion, we inserted a complementary DNA coding for a neomycin-resistance gene, including the stop codon. The resultant vector (Fig. 1a) was transfected into embryonic stem cells (D3), and G418-resistant clones were screened for homologous recombination by Southern blot analysis. Blastocysts from C57Bl/6 mice were injected with 10–16 targeted embryonic stem cells and implanted into pseudopregnant CD-1 foster mothers, and one of the chimeric (>60%) male pups was mated with C57Bl/6 females. Germline transmission of the *IRS-1* knockout allele in the agouti pups was confirmed by polymerase chain reaction (PCR) and Southern blotting (Fig. 1b, c). Heterozygous offspring were then bred to homozygosity. The absence of IRS-1 protein in the *IRS-1*^{–/–} mice was confirmed by western blotting of liver and muscle extracts (Fig. 1d and e). Although *IRS-1* is widely expressed, including in the developing fetus⁶, disruption of the *IRS-1* gene did not result in significant embryonic lethality. Genotyping of the mice generated by intercrossing the heterozygotes revealed a ratio of offspring of 23.4:53.2:23.4 for *IRS-1*^{–/–}, *IRS-1*^{+/–}, *IRS-1*^{+/+} mice, respectively.

Homozygous *IRS-1*^{–/–} mice showed a birth weight between 40 and 60% of that of the heterozygotes or homozygous unaffected littermates. The homozygous *IRS-1* knockouts grew in proportion to their smaller size and maintained body weights between 50 and 60% of wild-type littermates up to age 4 months (Fig. 1f). Histological examination of long bones at birth, two and four months of age, as well as differential staining of cartilage and bone in whole skeletons of neonatal mice, showed a normal progression of ossification in homozygous *IRS-1*^{–/–} mutants compared with control littermates. Adult *IRS-1*-knockout mice of both sexes were fertile and litter size was normal. Female *IRS-1*-knockout mice were also capable of nursing their young.

IRS-1^{–/–} mice were insulin- and IGF-1-resistant and showed abnormal glucose tolerance. Thus, although there was no significant difference in fasting glucose levels between wild-type *IRS-1*^{+/+}, heterozygote *IRS-1*^{+/–} and homozygote *IRS-1*-deficient mice (Fig. 2a), intraperitoneal glucose-tolerance tests in the *IRS-1*-knockout mice revealed significant levels of hyperglycaemia at 15–60 min compared with control (Fig. 2b); fasting plasma insulin levels were increased 2.3-fold in *IRS-1*^{–/–} mice ($P < 0.05$) (Fig. 2a). Furthermore, the hypoglycaemic response to insulin (Fig. 2c) and IGF-1 (Fig. 2d) in the *IRS-1*^{–/–} mice was significantly less than that in controls. This correlated with a marked reduction in insulin-stimulated glucose transport in isolated adipocytes (Fig. 2e).

Three of the earliest steps in insulin action are stimulation of the receptor kinase, phosphorylation of IRS-1, and binding of the 85K regulatory subunit of PI(3)K, leading to activation of its catalytic subunit. In control *IRS-1*^{+/+} mice, insulin injection resulted in a 5.7-fold increase of IRS-1-associated PI(3)K activity in liver (Fig. 3a and b, left) and a 28-fold increase in muscle (Fig. 3c, left) in anti-IRS-1 immunoprecipitates within 1–3 min. By contrast, in liver and muscle of *IRS-1*^{–/–} mice, there was no

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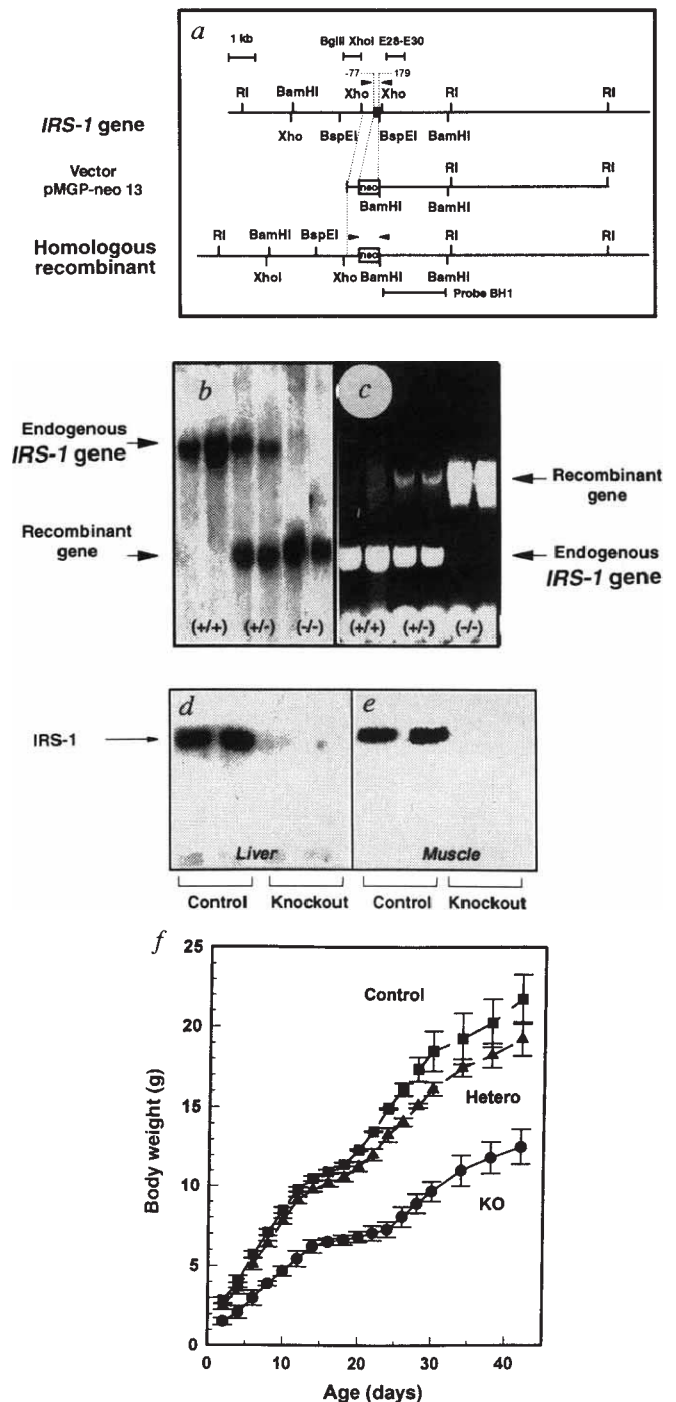
FIG. 1 Gene targeting of the *IRS-1* locus. **a**, Restriction map of the *IRS-1* gene (top), the targeting vector (middle) and the homologous recombinant gene (bottom). **b**, Southern blot analysis of DNA from homozygous mutant mice ($-/-$), heterozygotes ($+/-$) and wild-type animals ($+/+$). **c**, PCR analysis of genomic DNA. **d**, **e**, Western blot analysis of liver and muscle showing lack of IRS-1 protein in the *IRS-1* knockout mice. Each lane represents tissues from one animal and are representative of 3 experiments. **f**, Growth curves of *IRS-1* $^{+/+}$ (control), heterozygotes (hetero) and homozygous *IRS-1* $^{-/-}$ mutants (knockout, KO) on a C57Bl/6 $\times$ 129/Sv background are shown. Data are from two litters and are representative of 60 animals. Values are mean $\pm$ s.e.m. of three *IRS-1* $^{-/-}$, four *IRS-1* $^{+/+}$ and four *IRS-1* $^{+/-}$ animals.

METHODS. **a**, The mouse *IRS-1* gene containing the 5' flanking region, first exon and a part of the first intron was cloned as described⁵. An 8-kb fragment, containing the 5' flanking region and the first 2.6 kb of coding sequence was cloned into the *Eco*RI site of pBluescript (pBS, Stratagene). This plasmid was digested with *Bsp*EI (16 bp after the ATG codon) and treated with *Bal*31 to delete bases 16 to -77, including the ATG codon, from the 5' strand and bases +17 to +179 from the 3' strand. A promoterless neomycin-resistance (*neo*) gene fragment (bases 704 to 1,618; pMCneo polyA (Stratagene)) was inserted into the deleted region and this plasmid was named pMGP-neo3. To delete most of the promoter of the *IRS-1* gene, pMGP-neo3 was digested with *Bst*EII and treated with *Bal*31. This *IRS-1* fragment was then subcloned into the *Eco*RV and *Eco*RI site of pBS and renamed pMGP-neo6. The 7-kb *IRS-1* fragment containing the remainder of the coding sequence and the 3'-flanking region was subcloned into the *Eco*RI site in pMGP-neo6, and the resultant targeting vector was named pMGP-neo13. This vector consisted of bases -450 to +6 kb of the mouse *IRS-1* gene in which bases -77 to +179 had been deleted and replaced by the *neo* gene cassette. D3 embryonic stem cells were transfected with linearized pMGP-neo13 (ref. 29). Colonies resistant to G418 were analysed for homologous recombination by Southern blotting following digestion with *Eco*RI and *Bsp*EI, *Bam*HI, or *Bam*HI and *Bsp*EI, and hybridized with probe A (bases 1,879 to 2,549), probe B (*neo* gene); or probe C (bases -1,582 to -1,317). **b**, Chimaeric mice were generated according to ref. 29. Germline transmission of the *IRS-1*-knockout allele in agouti offspring was confirmed by polymerase chain reaction (PCR) of tail DNA using *neo* primers. **F**₁ heterozygous mice were intercrossed to produce homozygotes. Genotyping was also assessed by Southern blotting using *Bam*HI digestion and the probe BH1 (**a**). This generated a single 6-kb band in the presence of the endogenous *IRS-1* allele in *IRS-1* $^{+/+}$ mice, two bands of 6 kb and 2.5 kb in *IRS-1* $^{+/-}$ mice and a single band of 2.5 kb in *IRS-1* $^{-/-}$ mice³⁰. **c**, PCR was done using primers corresponding to bases -124 to -101 and 293 to 312 of the endogenous *IRS-1* gene, which flanked the *neo* cassette. This generated fragments of 1.25 kb for the recombinant *IRS* gene and 440 bp for the endogenous *IRS-1* gene. **d**, **e**, Liver homogenates (9 mg) immunoprecipitated with anti-IRS-1 antibody or total muscle homogenate (400 μ g) were solubilized, resolved on 6% SDS-PAGE and transferred to nitrocellulose. The blots were then probed with an anti C-terminus IRS-1 antibody and detected by ¹²⁵I-protein A¹⁵.

stimulation of IRS-1-associated PI(3)K activity, consistent with the absence of IRS-1 protein and inability of this pathway to participate in insulin signalling.

In contrast to this lack of activity in the IRS-1 precipitates, activation of PI(3)K by insulin was found in anti-phosphotyrosine immunoprecipitates of both liver and muscle from the IRS-1-deficient and control mice (Fig. 3, middle panels). Likewise, sequential immunoprecipitation of liver and muscle extracts of *IRS-1* $^{-/-}$ mice with anti-IRS-1 antibodies followed by anti-phosphotyrosine antibodies revealed considerable insulin-stimulated PI(3)K activity (2.4-fold for liver, 15-fold for muscle) (Fig. 3, right panels). These data suggest that non-IRS-1 tyrosine-phosphorylated protein(s) associate with PI(3)K following insulin stimulation.

To evaluate the mechanisms involved in insulin action and PI(3)K activation in IRS-1-deficient mice, the mice were injected



with insulin and the phosphotyrosine proteins in muscle and liver determined by immunoblotting of anti-phosphotyrosine, anti-IRS-1 and anti-p85 precipitates. As previously described¹⁵, phosphotyrosine blotting of muscle and liver immunoprecipitates from control animals following insulin stimulation demonstrated phosphorylation of the insulin receptor β -subunit ($M_r \sim 95K$) and IRS-1 ($M_r \sim 175K$) (Fig. 4a, d). The identity of the latter was confirmed by immunoprecipitation with anti-IRS-1 antibody (Fig. 4b, e), as well as by its ability to be co-precipitated with PI(3)K in anti-p85 precipitates (Fig. 4c, f). Extracts of muscle and liver from *IRS-1* $^{-/-}$ animals demonstrated insulin-receptor β -subunit phosphorylation, but in contrast to the controls, there was no distinct band in the region of IRS-1 in muscle, and in liver there was a tyrosine-phosphorylated band which migrated just above IRS-1 on SDS-PAGE (Fig. 4d). Furthermore, in both tissues no band corresponding to

FIG. 2 Blood glucose and plasma insulin (a), glucose tolerance tests (b), insulin/IGF-1 tolerance tests (c and d) and glucose uptake in adipocytes (e) in *IRS-1*^{-/-} and *IRS-1*^{+/+} mice.

METHODS. Blood glucose concentrations and plasma insulin levels (a) were measured between 8:00 and 10:00 a.m. after overnight fasting on 4–8-week old anaesthetized mice. Immunoreactive insulin concentrations in plasma were measured by radioimmunoassay. Insulin (c) and IGF-1 (d) tolerance tests were performed after a 6-hour fast on 6–16-week-old mice using an intraperitoneal injection of either 0.75 IU per kg human insulin or 1 mg per kg IGF-1 (Eli Lilly). Results are expressed as a percentage change from fasting blood glucose concentration and were not significantly different between groups. Glucose tolerance tests (b) were done on *IRS-1*-deficient and wild-type animals after a fasting for 6 h by intraperitoneal injection of 2 g D-glucose per kg at time zero. Data were obtained from at least 8 animals in each group, ranging in age from 5–12 weeks. Results are mean $\pm$ s.e.m. Asterisks in a–d: *, $P < 0.05$; **, $P < 0.025$; and ***, $P < 0.01$. Glucose transport was measured in isolated adipocytes (e). After a 4-hour fast, male mice (12–20 weeks old) were anaesthetized by intraperitoneal injection of 115 mg kg⁻¹ sodium pentobarbital (Abbott Laboratories). As soon as pedal and corneal reflexes stopped, the epididymal fat pads were removed. Adipocytes were isolated by digesting epididymal fat pads for 1 h in Krebs–Ringer HEPES (30 mM) buffer containing 1 mg ml⁻¹ collagenase (Worthington), 200 nM adenosine and 2.5% BSA (Sigma) as described³⁰. Cells ($\sim 10^5$ ml⁻¹) were incubated for 30 min in the absence or presence of human insulin. Glucose transport was then assayed for a further 30 min using 3 μ M universal ¹⁴C-glucose (286 mCi mmol⁻¹; Amersham). The reaction was stopped by spinning cells for 30 s through 100 μ l dinonyl phthalate. Microfuge tubes were cut through the oil layer, and the upper phase containing the cell layer was counted for incorporated radioactivity. Data are mean $\pm$ s.e.m. of four mice in each group in four independent experiments with triplicate determinations. **, $P < 0.025$.

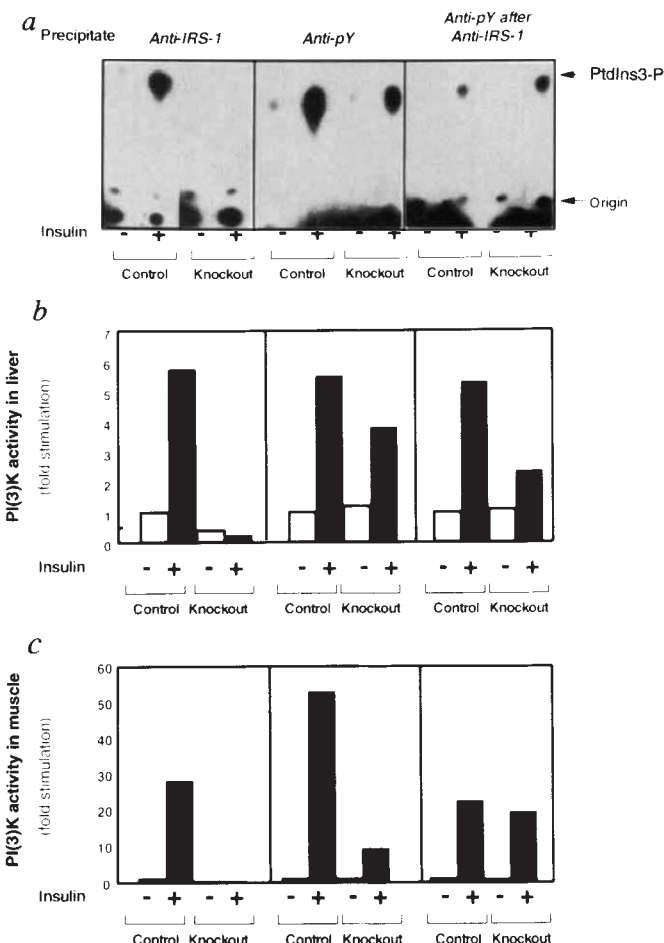
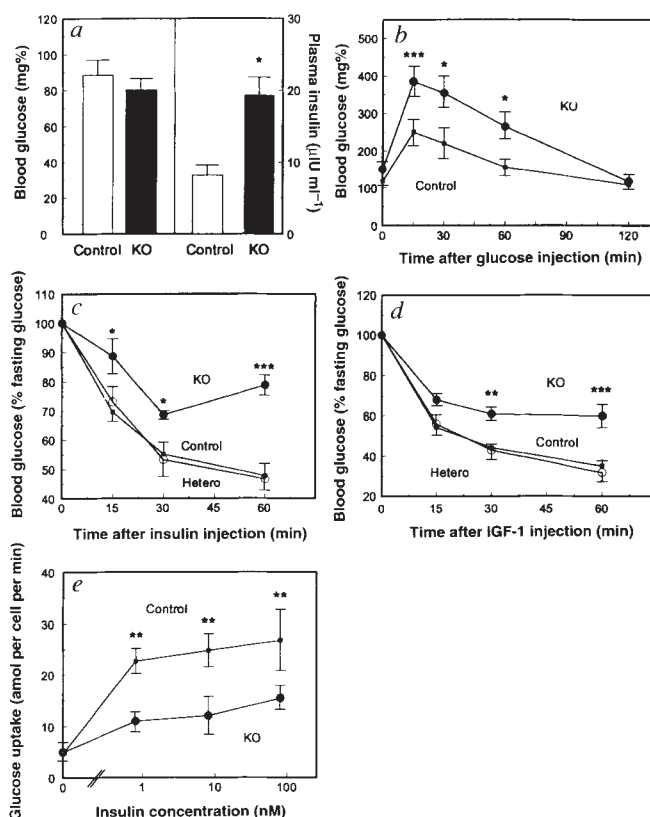


FIG. 3 Insulin-stimulated activation of PI(3)K in the *IRS-1*^{-/-} mouse. Supernatants of liver or muscle homogenates containing equal amounts of protein were immunoprecipitated in duplicate with the indicated antibody; immunoprecipitates were washed extensively and assayed *in vitro* for PI(3)K activity. **a**, Representative panel from an experiment using liver homogenates. **b**, Liver PI(3)K activity was assayed in supernatants of liver homogenates containing 5–10 mg total protein after immunoprecipitation with the indicated antibody. **c**, Muscle PI(3)K activity was measured in supernatants of homogenates containing 2.5–5 mg total protein following immunoprecipitation with the indicated antibody. For **b** and **c**, data are the mean of three separate experiments in seven *IRS-1*^{-/-} and six *IRS-1*^{+/+} mice and are expressed as fold stimulation of activity above that of the non-insulin-treated controls.

METHODS. Five units of regular human insulin (Lilly) were injected as a bolus into the inferior vena cava of anesthetized mice. The liver, gastrocnemius and quadriceps muscles were removed at 1, 2.5 and 3 min after insulin injection, respectively, and homogenized at 4 °C as described¹¹. Liver and muscle homogenates were allowed to solubilize at 4 °C for 1 h and clarified by centrifugation at 15,000 r.p.m. for 1 h. Supernatants containing equal amounts of protein were immunoprecipitated overnight with anti-IRS-1 C-terminal (6 μ g ml⁻¹), antiphosphotyrosine 4G10 (3 μ g ml⁻¹) (from M. White), anti-insulin receptor C-terminal (3 μ g ml⁻¹) or control rabbit serum. Immune complexes were collected with 120 μ l of a 50% slurry of protein A–Sepharose (Pharmacia). Supernatants of samples that were initially precipitated with anti-IRS-1 antibody were reprecipitated with anti-phosphotyrosine antibody in a similar manner. All immunoprecipitates were washed extensively; the PI(3)K reaction was performed as described¹¹. ³²P incorporation into phosphatidylinositol-3-phosphate (PtdIns-3-P) was quantified using a Phosphorimager (Molecular Dynamics).

tyrosine-phosphorylated IRS-1 was detected in anti-IRS-1 precipitates following insulin stimulation (Fig. 4b, e). But in immunoblots of anti-p85 precipitates, a faint band migrating just above the position of IRS-1 in controls was detected in the muscle of the knockout mice, which was suggestive of an alternative substrate (Fig. 4c). Analyses of anti-p85 precipitates from liver were even more striking and revealed a clear tyrosine-phosphorylated protein migrating ~10K above the position of IRS-1 (Fig. 4f, asterisk). The fact that this was not IRS-1 was confirmed by blotting of anti-p85 immunoprecipitates with IRS-1 C-terminal antibodies, which revealed p85-associated IRS-1 in liver extracts of normal mice, but not in extracts of *IRS-1*^{-/-} mice (Fig. 4g). Thus IRS-1-deficient mice use an alternative high-molecular-weight substrate of the insulin receptor which binds to PI(3)K, but does not react with anti-IRS-1 antibodies; we named this alternative substrate IRS-2.

Several other candidate lower-molecular-weight substrates of the insulin and IGF-1 receptor systems have been proposed from

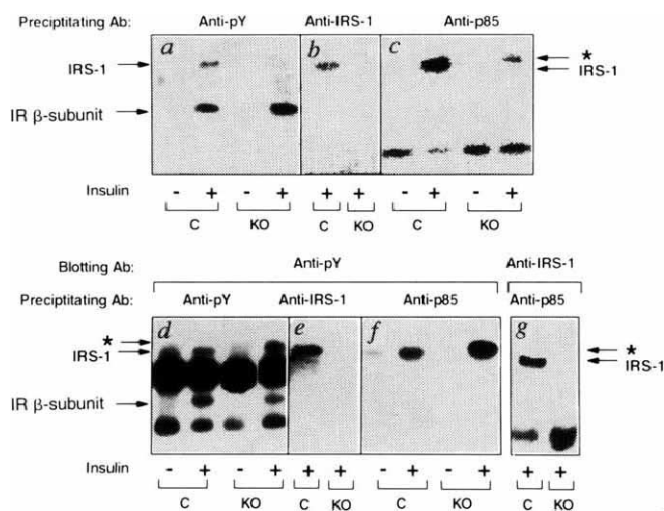


FIG. 4 Phosphotyrosine proteins in muscle and liver of *IRS-1*^{-/-} mice. a–c, Muscle homogenate (3.5 mg) from insulin-treated or control mice were immunoprecipitated with the indicated antibodies and subjected to reducing SDS-PAGE and western blotting with anti-phosphotyrosine antibodies. The migration of the tyrosine-phosphorylated protein (IRS-2) detected in anti-phosphotyrosine and anti-p85 immunoprecipitates from the IRS-1-knockout animal following insulin stimulation is indicated by the asterisk and is about 10K above that of IRS-1. Each lane represents tissue from one animal; each blot is representative of at least 3 experiments. d–g, Liver homogenate (9 mg) from insulin-treated or control mice was immunoprecipitated with the indicated antibodies and subjected to reducing SDS-PAGE and western blotting with anti-phosphotyrosine antibodies (d, e, f) or anti-IRS-1 antibody (g). Each lane represents tissue from one animal; each blot is representative of at least 3 experiments.

METHODS. Frozen liver and muscle samples previously collected at 1.5 and 3.5 min, respectively, after insulin injection were powdered and immediately homogenized in buffer containing 50 mM HEPES (pH 7.4), 50 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 1% Triton X-100, 10 µg ml⁻¹ aprotinin, 5 µg ml⁻¹ leupeptin, 2 mM benzamide and 2 mM PMSF. Homogenates were allowed to solubilize for 1 h at 4 °C before centrifugation at 55,000 r.p.m. for 1 h. Supernatants containing equal amounts of total liver or muscle protein were immunoprecipitated overnight with the indicated antibody. Immune complexes were collected with protein A-Sepharose, washed, solubilized in Laemmli sample buffer and separated using 6% SDS-PAGE. Proteins were transferred to nitrocellulose, probed with anti-phosphotyrosine or anti-IRS-1 C-terminal antibodies, detected with ¹²⁵I-labelled protein A, and visualized by autoradiography. Polyclonal antibodies to mouse p85 α -subunit of PI(3)K and the monoclonal antiphosphotyrosine antibody 4G10 were from M. White.

cell culture experiments, including the protein Shc, which like IRS-1 can bind to GRB-2 and activate Ras^{2,12}, and one or more proteins of 60–62K which may be phosphorylated in response to insulin and interact with PI(3)K and the GTPase-activating protein of Ras^{16,17}. Shc does not appear to be abundantly expressed in the usual target tissues for insulin action, and we found no phosphotyrosine-containing proteins of 60–62K in anti-p85 precipitates. In cultured cells PI(3)K also binds directly to the phosphorylated insulin receptor¹⁸, but we could not detect insulin receptor in our anti-p85 precipitates, or PI(3)K activity in anti-receptor immunoprecipitates (data not shown).

There is considerable evidence that IRS-1 is important for cellular signalling by the insulin and IGF-1 receptors^{2,3,9,13,19–22}. Our results and those of Tamemoto *et al.*²³ both show that IRS-1-deficient mice are insulin- and IGF-1-resistant and have marked retardation of intrauterine growth. They also indicate that IRS-1-independent signalling is significant, as shown by a sizeable residual action of these hormones to lower blood glucose in the animal, as well as a direct effect of insulin to stimulate glucose uptake in isolated adipocytes. Furthermore, although intrauterine growth retardation of *IRS-1*-knockout mice is comparable to mice deficient in IGF-1 or IGF-1 receptor, post-natal growth rates of the latter animals are severely retarded, development of ossification centres is delayed, perinatal lethality and gonadal hypoplasia is increased and, in the case of the receptor-knockout, there are severe changes in pulmonary, muscular and nervous system development which result in early death²⁴.

Two clues as to the nature of this alternative pathway lie in the PI(3)K studies and phosphotyrosine immunoblots. First, despite the absence of IRS-1-associated PI(3)K activity, there is still significant insulin stimulation of PI(3)K in anti-phosphotyrosine immunoprecipitates. Second, this stimulation of PI(3)K activity is accompanied by the appearance of the new insulin-stimulated phosphoprotein, IRS-2, which can be detected in anti-phosphotyrosine immunoblots of anti-p85 and anti-phosphotyrosine precipitates. IRS-2 has an apparent *M_r* ~10K larger than IRS-1, and does not blot or precipitate with antibodies against IRS-1, appearing to act as an alternative substrate of the insulin receptor and substitute for IRS-1 in binding to and activating PI(3)K pathways of post-receptor signalling. It remains to be determined whether or not IRS-2 activates other pathways.

The exact nature of IRS-2 is uncertain. Experiments with hepatoma cells²⁵ indicate that IRS-1 may be only one component of the pp185 band observed following insulin stimulation, and myeloid cells^{26–28} contain a protein of ~170K, termed 4PS, which is immunologically distinct from IRS-1 but may act as a substrate for the insulin receptor and an interleukin-4-linked tyrosine kinase activity. IRS-1 can also serve a substrate for the interleukin-4 related kinase, suggesting that 4PS and IRS-1 carry similar phosphorylation motifs²⁸. Until specific reagents for IRS-2 and 4PS are available, we cannot determine the precise relationship between these proteins. □

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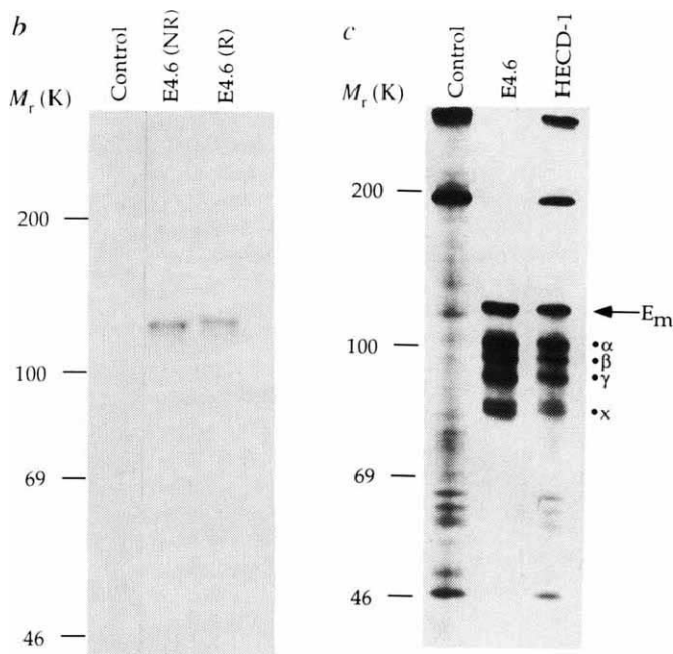
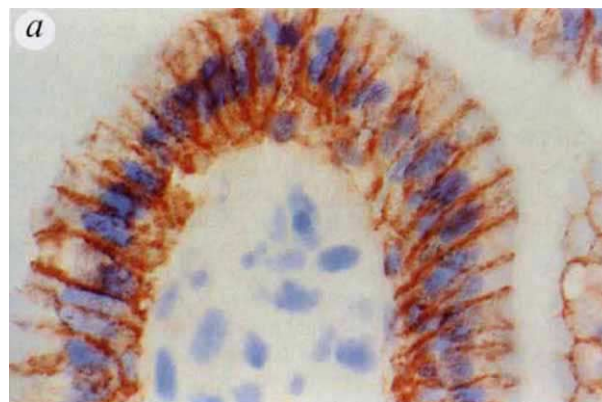


FIG. 1 Identification of the E4.6 antigen as E-cadherin. **a**, Sections of frozen human normal small intestine were stained with the E4.6 mAb. A single villus is shown. Note the prominent staining of the basolateral surface of the columnar epithelial cells. **b**, 16E6.A5 EpC monolayers were surface-labelled, solubilized, immunoprecipitated with control (T-cell receptor, TCR- $\delta 1$) or E4.6 mAbs, and analysed by SDS-PAGE under non-reducing (NR) or reducing (R) conditions. mAb E4.6 immunoprecipitated a single radiolabelled species of M_r 120K that increased to 123K under reducing conditions, indicating the presence of intrachain disulphide bonds. **c**, Metabolic labelling with [35 S]methionine and cysteine for 4 h followed by solubilization, immunoprecipitation with control (TCR- $\delta 1$), E4.6 or HECD-1 (ref. 6) mAbs, reduction with 2-mercaptoethanol, and analysis by SDS-PAGE revealed that the pattern of bands immunoprecipitated with E4.6 was indistinguishable from the pattern seen with HECD-1. The mature E-cadherin protein is designated E_m and the associated catenins¹² are labelled α , β and γ . A fourth co-precipitating band of unknown identity was also seen (x).

METHODS. **a**, The E4.6 mAb was produced by fusing spleen cells from an RBF/DnJ mouse immunized with 16E6.A5 cells with the P3 $\times$ 63Ag8.653 myeloma using standard procedures. Acetone-fixed sections (4 μ m) of frozen human normal small intestine mounted in OCT (Tissue-TEKTM) were stained with E4.6. Staining was visualized using the indirect immunoperoxidase method¹³. **b** and **c**, Confluent monolayers of 16E6.A5 cells were surface-labelled with [125 I] using lactoperoxidase as described¹⁴. For biosynthetic labelling, 16E6.A5 cells were labelled for 4 h with 1 mCi of [35 S]Express in methionine- and cysteine-free medium. Cells were solubilized in 1% Triton X-100. Lysates containing the equivalent of 1–2 $\times$ 10⁶ cells were immunoprecipitated with the indicated mAbs, and analysed by SDS-PAGE as described¹⁵.

Adhesion between epithelial cells and T lymphocytes mediated by E-cadherin and the $\alpha^E\beta_7$ integrin

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IN contrast to sessile cell types, lymphocytes migrate through the vasculature to become diffusely distributed in tissues or organized in lymphoid structures. A complex array of adhesion molecules including selectins, integrins and their counter-receptors mediate lymphocyte homing and migration into tissues and may be constitutively expressed or induced^{1,2}. However, the molecules that mediate the tissue-specific retention of lymphocytes within the parenchyma have not been identified. Along the epithelium at the basolateral surface of enterocytes, intestinal intraepithelial lymphocytes are found. These T cells of the mucosal immune system serve as a model for the tissue-specific compartmentalization of lymphocytes. We investigated whether the localization of these intestinal intraepithelial lymphocytes could be mediated by specific interactions between adhesion molecules expressed selectively on this subpopulation of T cells and tissue-restricted adhesion molecules on epithelial cells. Here we show that heterotypic adhesive interactions between epithelial cells and intraepithelial lymphocytes *in vitro* are mediated by E-cadherin and the $\alpha^E\beta_7$ integrin.

To identify a specific adhesion molecule on epithelial cells (EpC) that might mediate adhesion to intraepithelial lymphocytes (IEL), we immunized mice with a mucosal EpC line (16E6.A5) known to support divalent-cation-dependent IEL adhesion³ and screened the resultant hybridoma culture supernatants for the ability to inhibit IEL-to-EpC adhesion. One efficient inhibitor of IEL-to-EpC adhesion stained the basolateral surface

of intestinal EpC, the site to which IEL compartmentalize (Fig. 1a). Biochemical characterization of the E4.6 antigen on EpC revealed that it was E-cadherin. The E4.6 monoclonal antibody immunoprecipitated a mature cell-surface protein (E_m) of relative molecular mass 123,000 (M_r 123K) after surface labelling with ^{125}I (Fig. 1b), which associated with α , β and γ catenin after ^{35}S -metabolic labelling (Fig. 1c). The previously characterized E-cadherin monoclonal antibody HECD-1 immunoprecipitated a similar array of proteins that were found to be identical to those recognized by the E4.6 antibody, by two-dimensional SDS-PAGE/isoelectric focusing and by Cleveland digest peptide mapping studies (not shown).

The newly developed E4.6 antibody inhibited IEL-to-EpC adhesion by 68% (Fig. 2a, upper panel). To confirm the role of E-cadherin in IEL-to-EpC adhesion, a human E-cadherin complementary DNA⁴ was transfected into a murine fibroblast cell line (E-cadherin/L cell) that lacks endogenous expression of cadherins yet expresses the catenins necessary for classical cadherin function⁵. Fluorescence-activated cell sorting (FACS) analysis on L cells collected with 0.01% trypsin and 1 mM CaCl_2 demonstrated surface levels of E-cadherin with a mean fluorescence intensity (MFI) of 158 compared with a MFI of 5 for L cells transfected with vector alone (vector/L cell). In a representative experiment, cultured IEL adhered to E-cadherin/L cell 89%

better than to vector/L cell monolayers (Fig. 2b). In addition, the binding of IEL to E-cadherin/L cell was blocked by 48% with the anti-E-cadherin antibody E4.6, but not by control antibody or the anti-E-cadherin antibody HECD-1, known to inhibit EpC monolayer formation⁶ (Fig. 2). We produced another anti-E-cadherin monoclonal antibody, E6.1, which also blocked IEL-to-EpC adhesion, whereas the DECMA-1 and ECCD-2 antibodies did not (data not shown) suggesting that only certain anti-E-cadherin antibodies functionally inhibit this interaction. In contrast, the binding of IEL to vector/L cell monolayers was not inhibited by E4.6 (Fig. 2b).

As E-cadherin classically functions in homophilic (cadherin/cadherin) interactions⁷, IEL were analysed for surface expression of E-cadherin by FACS. No surface expression was seen using four different anti-E-cadherin antibodies: E4.6, HECD-1 (Fig. 3a), DECMA-1 or ECCD-2 (data not shown). Northern analysis revealed that IEL do not express messenger RNA for E-cadherin (Fig. 3b). Thus, the E-cadherin-mediated interaction between IEL and EpC must be heterophilic (E-cadherin/non-E-cadherin).

The $\alpha^E\beta_7$ integrin is expressed by >95% of IEL, but <2% of circulating T lymphocytes⁸, and mediates specific adhesive interactions between IEL and mucosal EpC^{3,9}. Whereas IEL grown in the presence of transforming growth factor TGF- β 1

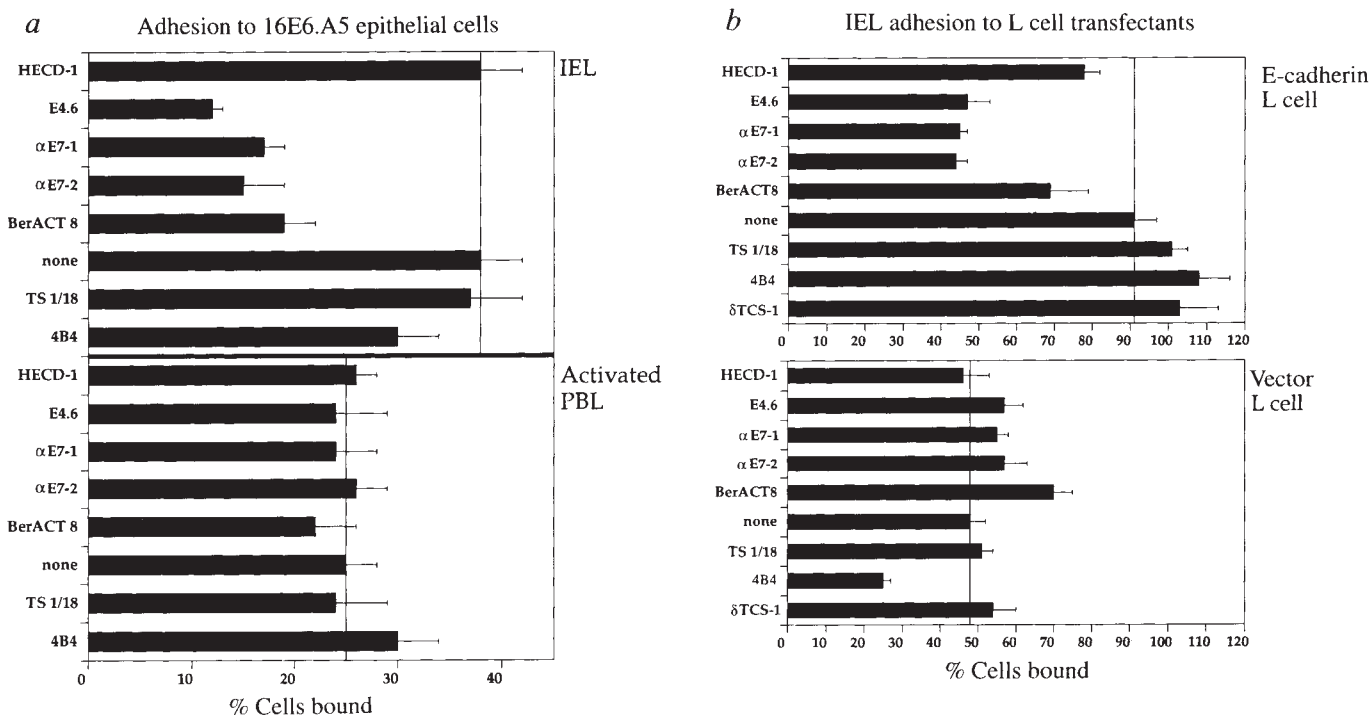


FIG. 2 IEL-to-EpC adhesion is mediated by E-cadherin and the $\alpha^E\beta_7$ integrin. **a**, Activated PBL grown in medium alone and IEL grown in medium containing 2 ng ml^{-1} TGF- β 1 were assayed for binding to confluent monolayers of the 16E6.A5 EpC line in the presence or absence of mAbs specific for $\alpha^E\beta_7$ (BerACT 8 (ref. 16); α E7-1 and α E7-2 (ref. 17); $\alpha^L\beta_2$ (TS 1/18 (ref. 18)), β_1 (4B4 (ref. 19)) and E-cadherin (E4.6, HECD-1). The E4.6 mAb as well as all three anti- $\alpha^E\beta_7$ mAbs blocked IEL-to-epithelial cell adhesion, but binding of activated PBL was not altered. One of three representative experiments is shown. **b**, Adhesion of IEL to L cells was enhanced by transfection of L cells with E-cadherin and this adhesion was blocked by mAbs recognizing $\alpha^E\beta_7$ and E-cadherin. IEL grown in the presence of 2 ng ml^{-1} TGF- β 1 were assayed for adhesion to monolayers of L cells (expressing E-cadherin or vector alone) in the presence or absence of mAb specific for E-cadherin (E4.6 or HECD-1), $\alpha^E\beta_7$ (BerACT 8, α E7-1 or α E7-2), $\alpha^L\beta_2$ (TS 1/18), $\gamma\delta$ TCR (δ TCS-1) or β_1 (4B4). The adhesion of IEL to vector/L cell monolayers

was blocked 48% with an anti- β_1 mAb, indicating that interaction with extracellular matrix components occurs in this system. Owing to a technical difference in quenching between the input fluorescence (in liquid buffer) and experimental fluorescence (measured after removal of buffer), more than 100% adhesion was observed as shown in the top panel. Experiment is representative of eight. Error bars indicate 1 s.d. and the solid vertical line denotes control binding in the absence of added antibody.

METHODS. T cell-to-EpC adhesion assays³ and IEL isolation and culture conditions^{10,20} have been described. Activated PBL were derived by stimulating mononuclear cells from peripheral blood with phytohaemagglutinin-P and irradiated feeder cells. The breast epithelial cell line 16E6.A5 was cultured as described³. The murine fibroblast L cell line (L-M, ATCC CCL1.3) was transfected with human E-cadherin in the pERF-1 vector²¹ or the pERF-1 vector alone using the calcium phosphate method.

express high levels of $\alpha^E\beta_7$, only a few per cent of peripheral blood lymphocytes (PBL) express $\alpha^E\beta_7$ (ref. 10). Both IEL and activated PBL bound to the incompletely polarized 16E6.A5 EpC monolayer, but the IEL adhered better by 34% (Fig. 2a). In a representative experiment, IEL were blocked from binding the 16E6.A5 EpC monolayer by 50–61% with three different antibodies that recognize the $\alpha^E\beta_7$ complex (BerACT-8, α -E7-

1 and α -E7-2), by 69% with the anti-E-cadherin E4.6, but not significantly with control antibody (Fig. 2a). In contrast, the adhesion of activated PBL to 16E6.A5 EpC was not inhibited by any antibody tested. These results demonstrated a correlation between the ability of E4.6 to block T-cell binding to EpC and expression of $\alpha^E\beta_7$ by the T cells and suggested that E-cadherin could be interacting with $\alpha^E\beta_7$.

FIG. 3 IEL do not express E-cadherin. a, IEL were stained for MHC class I (W6/32) and E-cadherin (E4.6, HECD-1) and analysed by FACS. Negative control staining (P3) is indicated by a filled histogram. Two additional anti-E-cadherin mAbs, DECMA-1 (Sigma) and ECCD-2 (Zymed) were also negative by FACS (data not shown). b, Total RNA (15 μ g per lane) was prepared from the 16E6.A5 EpC line (lane 1) and IEL grown in the absence (lanes 2 and 3) or presence of 2 ng ml⁻¹ TGF- β 1 (lane 4), size-fractionated, blotted onto nylon membrane and hybridized with a ³²P-labelled random-primed 0.8-kb *Bam*H1–*Xho*1 fragment of E-cadherin (top panel) or glyceraldehyde 3-phosphate dehydrogenase (Clontech) (bottom panel) as a control. The migration positions of 28S and 18S ribosomal RNA markers are indicated (top panel).

METHODS. FACS analysis was performed as described³. For northern blot analysis, total RNA was isolated from the indicated cell lines using LiCl (lanes 1 and 2) or guanidium isothiocyanate²² (lanes 3 and 4). About 15 μ g RNA from each sample were separated on a 1.5% agarose gel containing formaldehyde, transferred to nylon membrane and crosslinked with ultraviolet light. Hybridization with ³²P-labelled cDNA probes was performed for 16 h at 42 °C. After hybridization, the membrane was washed at a final stringency of 0.1 $\times$ SSC with 2% (w/v) SDS at 56 °C.

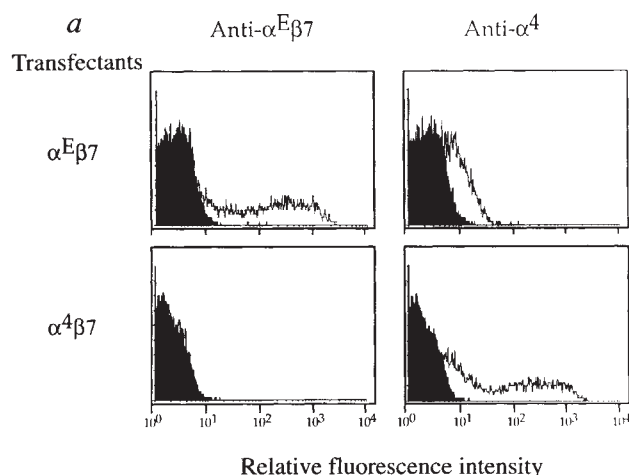
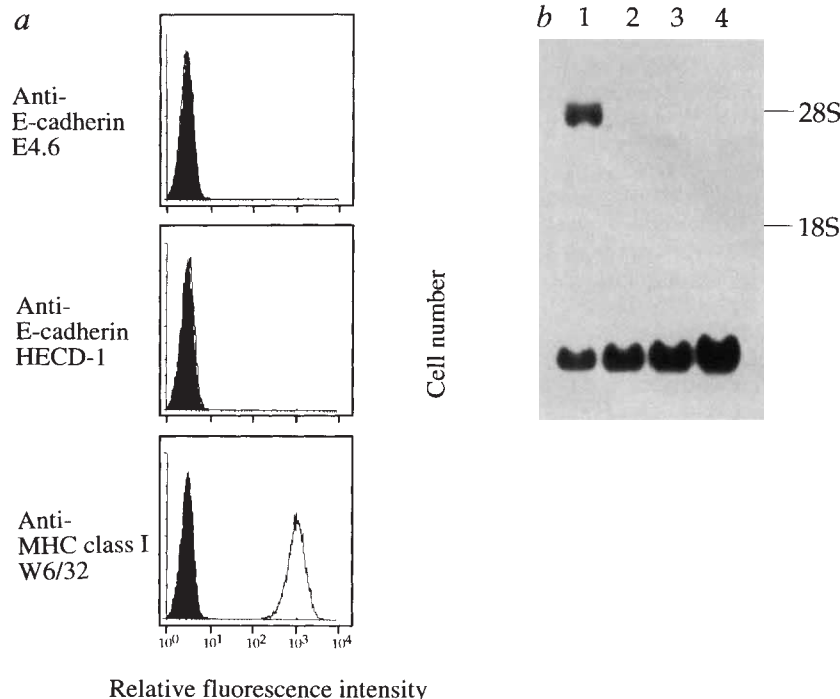
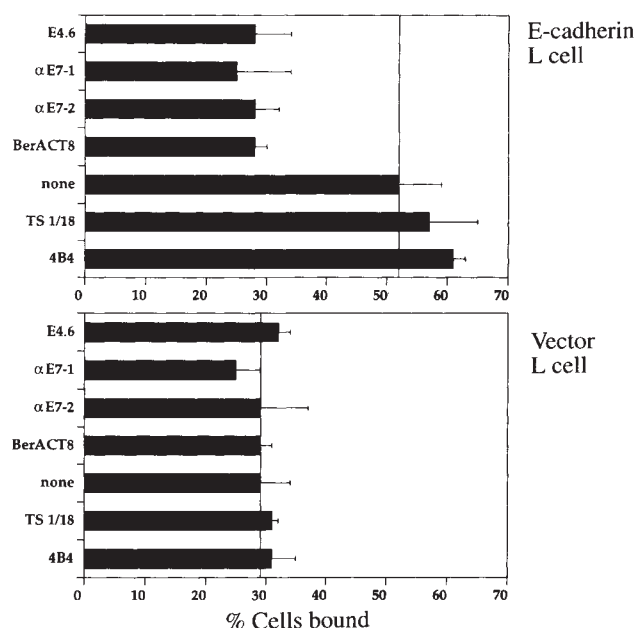


FIG. 4 Adhesion of COS-7 cells to L cells is enhanced by transfection of the COS-7 cells with $\alpha^E\beta_7$ and by transfection of L cells with E-cadherin. a, FACS staining of $\alpha^E\beta_7$ or $\alpha^4\beta_7$ transfected COS cells was carried out with control (P3, filled histogram), $\alpha^E\beta_7$ (BerACT8, open histogram) or α^4 (B5G10 (ref. 23), open histogram) mAbs. Control transfections with $\alpha^E\beta_7$ were assessed with mAb to α^4 which recognizes endogenous or transfected α^4 when paired with either endogenous β_1 or transfected β_7 . FACS analysis showed similar surface levels and per cent positive cells for $\alpha^E\beta_7$ and α^4 on COS-7 cells transfected with $\alpha^E\beta_7$ and $\alpha^4\beta_7$ respectively. The per cent of α^4 paired with β_1 versus β_7 was not determined; however, the level of endogenous β_1 was similar on the $\alpha^E\beta_7$ (MFI, 268) and $\alpha^4\beta_7$ (MFI, 230) transfectants. b, Adhesion of COS-7 cells transfected with $\alpha^E\beta_7$ to monolayers of L cells transfected with E-cadherin or vector alone was done in the presence or absence of mAb specific for E-cadherin (E4.6), $\alpha^E\beta_7$ (BerACT 8, α E7-1 or

b COS $\alpha^E\beta_7$ transfectant adhesion to L cell transfectants



α E7-2), $\alpha^L\beta_2$ (TS 1/18) or β_1 (4B4). One of five representative experiments is shown. c, COS-7 cells transfected with $\alpha^E\beta_7$ or $\alpha^4\beta_7$ also were assayed for adhesion to confluent monolayers of L cells transfected with E-cadherin in the presence or absence of mAb specific for E-cadherin (E4.6, ECCD-2 (Zymed) or HECD-1), $\alpha^E\beta_7$ (BerACT 8, α E7-1 or α E7-2), $\alpha^L\beta_2$ (TS 1/18) or β_1 (4B4). One of four representative

To investigate further any role for $\alpha^E\beta_7$ in E-cadherin-mediated adhesive events, COS-7 cells were transiently transfected to express similar levels of human $\alpha^E\beta_7$ ($\alpha^E\beta_7$ /COS) or $\alpha^4\beta_7$ ($\alpha^4\beta_7$ /COS) (Fig. 4a) and used in binding assays to monolayers of E-cadherin/L cell or vector/L cell. The $\alpha^E\beta_7$ /COS bound 79% better to E-cadherin/L cell than vector/L cell monolayers (Fig. 4b). The binding of $\alpha^E\beta_7$ /COS to E-cadherin/L cell was blocked by E4.6 (46%) and three different antibodies that recognize $\alpha^E\beta_7$ (46–52%) to the level of adhesion observed with vector/L cell. Monoclonal antibodies against β_1 or β_2 integrins did not inhibit this adhesion. $\alpha^E\beta_7$ /COS bound 81% better than control $\alpha^4\beta_7$ /COS to an L-cell/E-cadherin monolayer (Fig. 4c). Again, inhibition of binding by anti-E-cadherin and anti- $\alpha^E\beta_7$ antibodies was seen only when both molecules were expressed on their respective transfectants. Thus, expression of $\alpha^E\beta_7$ but not $\alpha^4\beta_7$ conferred increased adhesion of COS-7 transfectants to L cells only when E-cadherin was expressed. It seems unlikely that transfection of E-cadherin or $\alpha^E\beta_7$ into various different recipient cells induced the expression of unidentified molecules which functioned as counter-receptors. Thus the ability to transfect either $\alpha^E\beta_7$ or E-cadherin into a new cell type and confer increased adhesive ability on that cell only when both molecules are present suggests that these two molecules are counter-receptors.

Heterotypic interactions involving homophilic E-cadherin/E-cadherin binding have been observed between keratinocytes and Langerhans cells¹¹. As the T lymphocytes shown here to be involved in E-cadherin-mediated EpC binding do not express E-cadherin, this to our knowledge is the first description of a heterophilic interaction of E-cadherin, possibly with a member of the integrin family of adhesion molecules. The interaction of $\alpha^E\beta_7$ on IEL with E-cadherin on epithelial cells may serve as a fundamental mechanism for the retention and/or function of IEL within the epithelium, one site where lymphocytes are compartmentalized in a tissue-specific manner. Other cadherins may

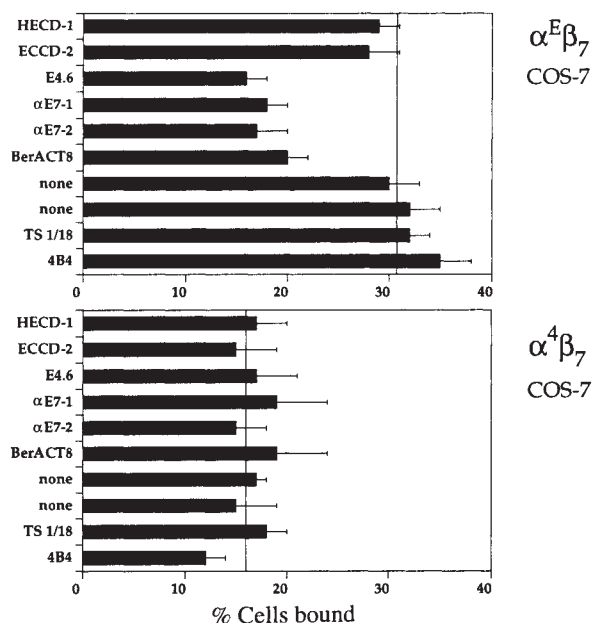
also mediate adhesion with leukocytes to confer tissue-specific localization having relevance to immune surveillance. □

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C Adhesion to E-cadherin transfected L cells



experiments is shown. Error bars indicate 1 s.d. and the solid vertical line denotes control binding in the absence of added antibody.

METHODS. COS-7 cells were transfected transiently using DEAE-dextran²⁴ with $\alpha^E\beta_7$ (refs 25, 26) or $\alpha^4\beta_7$ (refs 26, 27) and collected 72 h post-transfection with trypsin and EDTA for FACS analysis and use in adhesion assays. FACS analysis and adhesion assays were performed as described³.

mRNAs can be stabilized by DEAD-box proteins

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EUBACTERIAL messenger RNAs are synthesized and translated simultaneously; moreover the speed of ribosomes usually matches that of RNA polymerase^{1,2}. We report here that when in *Escherichia coli* the host RNA polymerase is replaced by the eightfold faster bacteriophage T7 enzyme for the transcription of the *lacZ* gene, the β -galactosidase yield per transcript is depressed 100-fold. But the overexpression of DEAD-box proteins³ greatly improves this low yield by stabilizing the corresponding transcripts. More generally, it stabilizes inefficiently translated *E. coli* mRNAs. Ribosome-free mRNA regions, such as those lying behind the fast T7 enzyme or between successive ribosomes on inefficiently translated transcripts, are often unstable⁴ and we propose that DEAD-box proteins protect them from endonucleases. These results pinpoint the importance of transcription-translation synchronization for mRNA stability, and reveal an undocumented property of DEAD-box RNA helicases. These proteins have been implicated in a variety of processes involving RNA⁵ but not mRNA stability.

We constructed a hybrid operon consisting of the *lacZ* gene followed by the gene encoding transfer RNA^{Arg5}, a rare *E. coli* arginine isoacceptor. We have shown that this tRNA is quantita-

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tively recovered from the transcript, so that its accumulation reflects the transcription frequency⁶. The hybrid operon is fused either to the T7 gene 10 promoter (P_{T7}), or to a typical *E. coli* promoter, that of the *lac* operon (P_{lac}), in such a way that the sequence transcribed from either promoter is identical. These constructs are then transferred as a single copy onto the chromosome of a Lac^- strain that expresses the T7 RNA polymerase (Fig. 1). We use ' P_{T7} ' or ' P_{lac} ' to indicate the resulting strains, as well as the corresponding transcripts and mRNA patterns. When grown to saturation, P_{T7} cells accumulate about 10-fold more $\text{tRNA}^{\text{Arg5}}$ than P_{lac} cells, consistent with the superior strength of the P_{T7} promoter. At the same time, the accumulation of β -galactosidase is 10-fold lower, representing a 100-fold reduction in protein yield per transcript (Fig. 2, control lanes). Therefore, either the stability or the translatability of P_{T7} transcripts is dramatically impaired.

Genes encoding DEAD-box proteins are ubiquitous in nature and they have been implicated in a variety of post-transcriptional processes⁷. Derivatives of pUC18 bearing either of two *E. coli* DEAD-box protein genes, *deaD*⁸ and *srnB*⁹, were introduced into P_{lac} and P_{T7} cells, resulting in the overproduction of the corresponding products (Fig. 2a) without affecting cell growth. In P_{lac} cells, this overproduction caused no change in the expression of β -galactosidase or reporter tRNA (Fig. 2). In contrast, in P_{T7} cells, the β -galactosidase expression was stimulated 25- and 30-fold by the *SrnB* and *DeaD* proteins, respectively. Meanwhile, the tRNA expression, and hence the transcription frequency, remained unchanged. Therefore, the *DeaD* and *SrnB*

proteins cause a large increase in the β -galactosidase yield per transcript, now only three- to fourfold lower than in P_{lac} cells. Measurements on exponentially growing cultures yielded similar results, but the increase in yield was only five- to tenfold, presumably because the *DeaD* and *SrnB* proteins accumulate mostly during late log phase¹⁰. These experiments were repeated with two other DEAD-box protein genes, the *E. coli dbpA* gene¹¹ and the complementary DNA encoding the human antigen p68 (not shown)¹². Although with the constructions used the *DbpA* product was substantially expressed and p68 was undetectable, only the latter stimulated β -galactosidase expression in P_{T7} cells, suggesting that individual DEAD-box proteins are specific in this respect.

Two control experiments clarified the significance of these findings. First, we removed the tRNA from our constructs. This did not affect the low β -galactosidase yield from the P_{T7} transcript or its stimulation by the *DeaD* product, showing that these effects are unrelated to the artificial presence of the excisable tRNA on the transcript (Fig. 3). Second, we removed a stretch of 24 amino acids from the *DeaD* protein, including the conserved motif His-Arg-Ile-Gly-Arg-X-X-Arg which has been

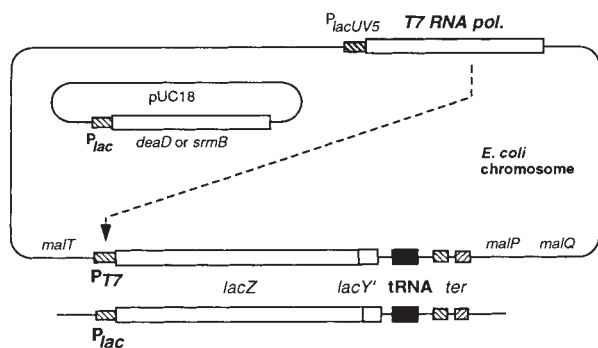


FIG. 1 Overall design of the experimental system. The *lacZ* gene followed by the first third of the *lacY* gene (white boxes), is fused to the *E. coli* $\text{tRNA}^{\text{Arg5}}$ gene (black box). For construction purposes, the genuine *lacZ* RBS has been replaced by the nearly equivalent¹⁵ *lamB* RBS. The tRNA carries a point mutation which allows it to be distinguished from the natural $\text{tRNA}^{\text{Arg5}}$ by hybridization. The *lac*- $\text{tRNA}^{\text{Arg5}}$ operon is fused downstream of either the T7 gene 10 promoter (P_{T7}) or the *lac* operon promoter (P_{lac}). The constructs end with two tandemly arranged terminators (*ter*), the major T7 late terminator and the rho-independent *trpA* terminator, 4.3 kb downstream of the promoter. These constructs are then inserted onto the *malA* chromosomal region of a Lac^- derivative of BL21(DE3)²⁵. This strain carries the T7 RNA polymerase gene (white box) under the control of the *lac UV5* promoter. In the final strains (P_{lac} and P_{T7}), we introduced derivatives of the pUC18 plasmid (pUC-*deaD* and pUC-*srnB*) bearing the *deaD* or *srnB* genes.

METHODS. Strains ENS133 (P_{lac}) and ENS134 (P_{T7}) are described elsewhere⁶. The plasmid pUC-*deaD* carries the *BclI*-*NsiI* fragment from pB13-1²⁶ inserted between the *Bam*HI and *Pst*I sites of pUC18. This fragment encompasses the *deaD* reading frame⁸ and 10 and 332 nucleotides (nt) of upstream and downstream flanking regions, respectively. The *DeaD* product starts at the third AUG in this reading frame⁸, as determined by Edman degradation. pUC-*srnB* carries a *Sall*-*Bam*HI insert encompassing the *srnB* coding sequence and 173 and 459 nt of downstream and upstream flanking sequences, respectively, including the putative *srnB* promoter. This fragment was initially cloned in pACYC184⁹.

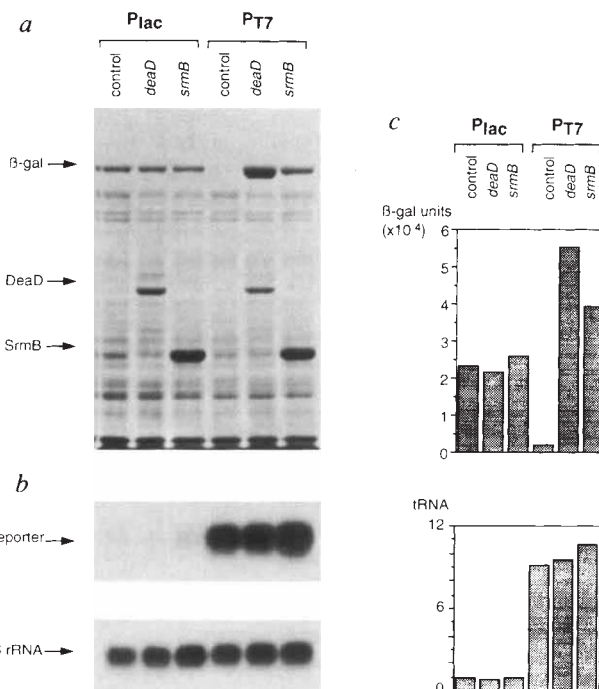


FIG. 2 The defect in β -galactosidase yield from P_{T7} transcript is alleviated by DEAD-box proteins. **a**, Coomassie-stained SDS-polyacrylamide gel showing the effect of an overproduction of the *DeaD* and *SrnB* products on protein expression from the P_{lac} (left) and P_{T7} (right) strains. 'Control', Extracts of cells harbouring the parental plasmid pUC18. The position of the β -galactosidase, *DeaD* and *SrnB* polypeptides are arrowed. The apparent M_r of the *DeaD* and *SrnB* products are ~64 and 51K, respectively. **b**, upper, Northern blot showing the expression of the transcriptional reporter tRNA in the extracts used in **a**. Lower, The same membrane was stripped and re-probed with a 5S rRNA probe. **c**, Diagrams showing the expression of β -galactosidase (upper) and reporter tRNA (lower) in the experiment shown in **a** and **b**. The β -galactosidase expressions are estimated from the activities in the extracts (in nmol ONPG hydrolysed per min per mg of proteins). The tRNA expressions are deduced from hybridization signals, using the 5S signal for calibration. They are normalized to the 'control' P_{lac} value (=1). METHODS. P_{lac} and P_{T7} cells transformed with plasmids pUC18, pUC-*deaD* or pUC-*srnB* were grown to saturation in rich synthetic medium⁶ containing ampicillin (0.1 mg ml⁻¹). The $\text{tRNA}^{\text{Arg5}}$ and 5S rRNA expressions and β -galactosidase activities were quantified as in ref. 6.

implicated in RNA binding¹³. The shortened product accumulates in P_{lac} and P_{T7} cells like the intact DeaD protein, but is much less efficient in stimulating β -galactosidase expression in P_{T7} cells (Fig. 3). This suggests that stimulation requires a direct interaction between the DeaD product and an RNA, plausibly the P_{T7} transcript itself.

We next used northern blots to analyse the P_{T7} and P_{lac} transcripts in exponentially growing cultures. Aside from a smear of growing and decaying molecules, the transcript pattern consists of the full-length 4.2 kilobase (kb) species and a pro-

cessed 3.2 kb species corresponding to the *lacZ* mRNA; the latter is not observed in the absence of translation¹⁴. In the P_{T7} strain, these species, and particularly those of 4.2 and 3.2 kb, were more abundant when the DeaD or SrmB products were overexpressed (Fig. 4a). Because the transcription frequency is unchanged (Fig. 2b), this higher abundance implies a higher transcript stability. DEAD-box proteins might affect this stability directly; alternatively, they might primarily improve translation, because translation and stability are interdependent for the *lacZ* mRNA¹⁵. To settle this ambiguity, we used a deriva-

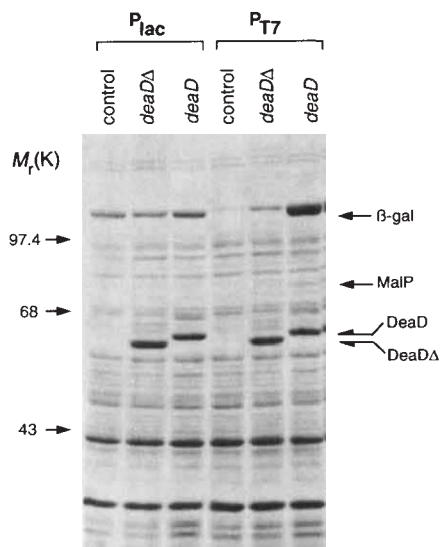
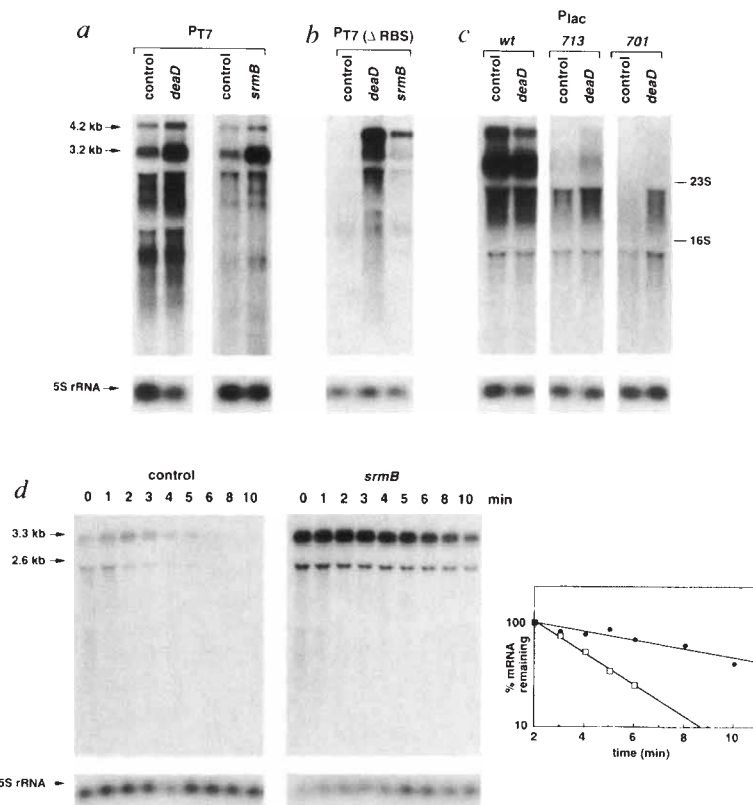


FIG. 3 The stimulation of P_{T7} expression by DEAD-box proteins involves their RNA-binding activity. Coomassie-stained SDS-polyacrylamide gel comparing the effect of the overproduction of the intact DeaD protein, or of a shortened product lacking the putative RNA-binding domain (DeaDA), on the expression of β -galactosidase in P_{lac} (left) and P_{T7} (right) strains. These strains differ from those described in Fig. 1 in lacking the *tRNA^{Arg5}* gene. The P_{T7} strain also lacks the *trpA* terminator located downstream of the major late T7 terminator, which increases the readthrough transcription of the downstream *malP* gene^{14,27} (Fig. 1). Note then that the MalP product, like β -galactosidase, is overexpressed in the presence of the DeaD product (rightmost lane).

METHODS. The strains used here are described elsewhere (IM3 and IM4 in ref. 16, renamed ENS33 and ENS34). Plasmid pUC-deaDA lacks the *Bss*HII-EcoRV fragment of pUC-deaD encompassing the DeaD residues 322 to 345. Cell growth and protein analysis are as in Fig. 2. The MalP product was identified by its comigration with the major 80K maltose-inducible protein from the parental BL21(DE3) strain (O. Raibaud, personal communication).

FIG. 4 DEAD-box proteins stabilize mRNAs. a, Northern blot analysis of the *lacZ* mRNA in P_{T7} cells containing either the pUC18 (control), pUC-deaD (*deaD*), or pUC-srmB (*srmB*) plasmids. b, As in a, except that the strain used (P_{T7} - Δ RBS) lacks a RBS in front of *lacZ*. c, As in a, except that the P_{lac} strain and two of its derivatives harbouring mutations that depress translation initiation were used. d, Northern blots illustrating the decay of the *thrS* transcript in P_{lac} cells harbouring pUC18 (control), or pUC-srmB (*srmB*). At zero time, exponentially growing cells were treated with rifampicin (0.2 mg ml⁻¹) and at subsequent times (min) aliquots of the cultures were collected and processed for northern blot analysis. The right panel illustrates the decay of the 3.3 kb *thrS* transcript in control (squares) and '*srmB*' (points) panels ($t_{1/2}$ = 1.7 and 7 min, respectively). A 2 min delay was allowed after rifampicin addition for the completion of nascent transcripts. In a-c, the full-length transcript (4.2 kb) and the processed *lacZ* mRNA (3.2 kb) are arrowed. Positions of the 16S and 23S rRNAs are also noted. In d, the two *thrS* species (3.3 and 2.6 kb) differ at their 3' ends²⁸. The strip below each autoradiogram shows the reprobing of the corresponding membrane with the 5S probe.

METHODS. Growth conditions as in Fig. 2, except that cultures were collected at an A_{500} of ~0.5 and that, in d, amino acids were omitted to increase *thrS* repression²¹. The P_{T7} strains used in a, and the P_{lac} strain used in c and d are as in Fig. 3; all other strains are isogenic to them except for the following changes. The P_{T7} - Δ RBS strain (IMO in ref. 29, renamed ENS32) differs from P_{T7} by a 51 bp deletion encompassing the *lamB* RBS, which abolishes β -galactosidase expression. P_{lac} derivatives carrying the 713 and 701 mutations (named ENS37 and ENS39) were obtained by replacing the *lamB* RBS by its 713 and 701 point-mutated alleles¹⁴. Membrane preparation and hybridization were as in ref. 15. The *lacZ* probe (Genebank Ecolac 2349 to 4176) and the *thrS* probe (Genebank Echthrin 161 to 669) encompass the central and 5' regions of the corre-



sponding genes, respectively. Except for the two experiments shown in a, the intensity of all autoradiograms are comparable.

tive of the P_{T7} strain (P_{T7} - Δ RBS) lacking a ribosome binding site in front of *lacZ*. Though untranslated, the *lacZ* gene is still efficiently transcribed in this strain because the T7 RNA polymerase is insensitive to polarity¹⁴. The transcript pattern was faint in this case, showing that the *lacZ* mRNA is unstable in the absence of translation; however, it was strongly enhanced in the presence of overproduced DEAD-box proteins, indicating a marked stabilization (Fig. 4b). We conclude that DEAD-box proteins stimulate the β -galactosidase expression from P_{T7} by stabilizing the corresponding transcripts.

The T7 RNA polymerase runs ~eightfold faster than the *E. coli* polymerase or ribosomes over the *lacZ* gene, creating a large ribosome-free mRNA region behind itself¹⁶. In particular, by the time of the first ribosome binding, a much longer stretch of naked RNA has been synthesized by the T7 than by the *E. coli* RNA polymerase. Now, mRNAs that are partially devoid of ribosomes are often unstable in bacteria⁴, especially if their 5' region is ribosome free^{17,18}. We hypothesized that P_{T7} transcripts are particularly unstable because of this unique mode of synthesis, and that DEAD-box proteins stabilize them by protecting their naked regions. Were this view correct, then those *E. coli* RNA polymerase transcripts that are unstable because of an inefficient translation should also be stabilized by DEAD-box proteins. The following observations support this view.

The P_{lac} transcript pattern, and hence stability, are unaffected by DEAD-box proteins (Fig. 4c). Two point mutations were used to depress the translation of this transcript. One (713) hits the Shine-Dalgarno element (SD), whereas the other (701) creates a secondary structure sequestering this element. We reported previously¹⁵ that, within the genuine *lacZ* gene, these mutations depress translation without affecting transcription; moreover, as a consequence of the translation-stability relationship, they destabilize the transcripts, most of which now decay before reaching full length. Consistently, the mutations weakened the P_{lac} transcript pattern, especially in the higher molecular mass range. The overproduction of the DeaD product reversed this effect, restoring a pattern more like that of the wild-type P_{lac} (Fig. 4c). Hence, DEAD-box proteins stabilize P_{lac} transcripts lacking a functional SD. Genes naturally lacking such an element are not uncommon in *E. coli*, and for one of them (*malT*¹⁹) we checked that overexpressed DEAD-box proteins strongly stabilize the corresponding mRNA (not shown).

Like many components of the protein-synthesizing machinery, the product of the *thrS* gene, threonyl-tRNA synthetase, binds its own mRNA and represses its translation^{20,21}. As for most similar cases, including ribosomal protein mRNAs⁴, the repression destabilizes the *thrS* transcript (M. Springer, personal communication). Compared with control, cells overproducing the DeaD or SrmB products accumulated the *thrS* transcript (Fig. 4d). By monitoring the transcript decay on northern blots after rifampicin addition, we showed that this accumulation stems from a marked stabilization (Fig. 4d). Interestingly, mutations have been characterized that prevent repressor binding, resulting in a constitutively high *thrS* translation ('O^c' mutations²¹). In a strain carrying such mutation, the level of the *thrS* transcript, and hence its stability, were not increased by DEAD-box proteins (not shown).

The decay of most *E. coli* mRNAs, including the *lacZ*¹⁵ and *thrS* (O. Yarchuk and M.D., unpublished results) transcripts, is controlled, presumably directly²², by RNase E. Conceivably, overexpressed DEAD-box proteins hinder RNase E action by binding directly to its cleavage sites or by unwinding the surrounding mRNA structure²³. Our results also show that the stability of certain mRNAs requires either a tight synchronization between transcription and translation, or the overproduction in the same cell of RNA-binding proteins such as DEAD-box helicases. In several expression systems, the gene of interest, borne on a multicopy plasmid, is transcribed in *E. coli* by the T7 RNA polymerase^{24,25}. We speculate that for some target genes the effectiveness of these systems depends on the presence

of cellular DEAD-box proteins, and can in turn be improved by their overproduction. □

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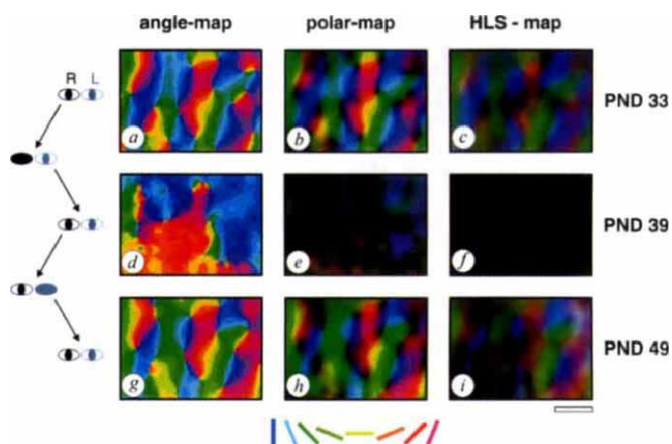
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ERRATUM

Reverse occlusion leads to a precise restoration of orientation preference maps in visual cortex

Dae-Shik Kim & Tobias Bonhoeffer

Nature **370**, 370–372 (1994)



As a result of a printing error, the colour code for the angle maps, polar maps and HLS maps in Fig. 2 was omitted. The correct figure is shown here, with the coloured bars at the bottom indicating how orientation preference is translated into colour. □

Artificial retinas — fast, versatile image processors

Kazuo Kyuma, Eberhard Lange, Jun Ohta, Anno Hermanns, Bryan Banish and Masaya Oita

Artificial retinas combine video camera and image processing functions, allowing machines to function in their environment with unprecedented autonomy, or to augment quality control, surveillance and hazard monitoring. We review several retina devices and reveal how they execute basic manipulations of the image at processing speeds well beyond the capabilities of the human eye.

RECOGNITION and understanding of real-world images is one of the most important information processing technologies for the 21st century. Such images contain a very large amount of information which present image processing systems cannot analyse in real time. This is because present systems separate image sensing and image processing: images are sensed by a camera and processed by a computer. Consequently, performance is limited by slow camera frame rates and low transmission rates between camera and computer. By contrast, human beings can execute very difficult tasks in real time. For example, we can instantly recall the names of people by glancing at their faces, and can analyse motion information by selectively detecting a moving object. This is due to the inherent parallelism of our visual system, particularly of our retina, which combines image sensing and processing functions.

The artificial retinas described in this article, having evolved out of a continued research effort within our laboratory to explore and develop optical neural devices¹⁻³ capable of on-chip image processing, are devices that can simultaneously sense and process images. To date, several kinds of related devices have been developed, such as vision chips, focal plane processors, and silicon retinas⁴⁻⁶. However, the specialized functions of the devices reported so far, such as spatial and temporal filtering, have precluded them from use in large-scale industrial or commercial applications. Therefore, devices with more 'on-chip' functionality are required. The most notable distinction between our artificial retinas and other devices is their on-chip functional flexibility.

Device, function, applications

Figure 1 shows our artificial retina (RETINA2) which consists of a two-dimensional (2-D) array of variable sensitivity photodetectors (VSPDs)^{7,8}. The VSPDs were formed by a side-by-side pair of diodes integrated onto and separated by a semi-insulating GaAs layer (pn-np structure). The size of each VSPD is $80 \times 80 \text{ mm}^2$, and the total size of a 128×128 array is $10.24 \times 10.24 \text{ mm}^2$.

The VSPD combines three devices:

photodetector, spatial light modulator (SLM), and analog memory^{3,9}. The photodetector current depends, both in sign and magnitude, on applied voltage, thus integrating detector and SLM into one variable sensitivity device. The analog memory effect encountered in these VSPDs stores conductivity information when a voltage is applied in the presence of an optical write pulse. This information can then be retrieved by injecting an optical readout pulse.

Image processing in RETINA2 is based on optical matrix-vector multiplication⁸. In Fig. 1, the input image is directly projected onto the chip as the weight matrix W . All VSPDs have one electrode connected along rows, yielding a sensitivity control vector S . Thus, the VSPD sensitivities can be set to arbitrary values at each row within a certain range. In addition, the remaining VSPD electrode is connected along columns, yielding an output current vector J defined by the matrix vector product $J = WS$.

The distinguishing feature of RETINA2 over other devices is the variety of processing that can be achieved through simply changing the control voltage pattern, S . Several examples of processing are summarized in Fig. 2 including TV camera-like image sensing, edge extraction, noise elimination (image smoothing), Fourier transform, pattern matching, and image compression/recognition. Two examples follow.

The first case is the edge-extraction operation shown in Fig. 1. The sensitivities of two adjacent detector rows are set to $+1$ and -1 , respectively, whereas all other sensitivities are set to 0. In this case, the output current is proportional to the difference in light intensities of the two active rows. By shifting the control voltage pattern cyclically ($0, +1, -1, 0, 0, \dots$), the horizontal

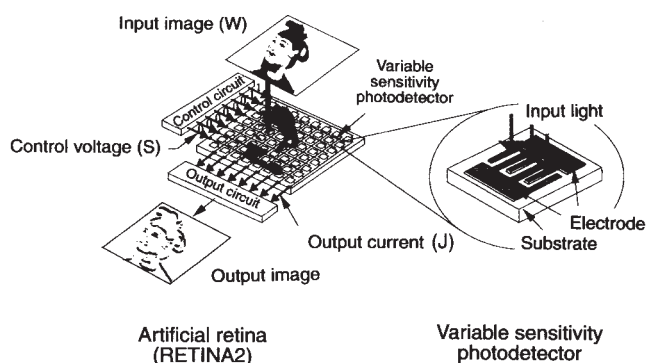


FIG. 1 Image processing in RETINA2 as a matrix-vector multiplication. The input image modulates the conductivities at the VSPD sites, thus generating a weight matrix W . This matrix is addressed with a control signal vector S , which also determines the operating mode of the retina. The resulting photocurrents are read out by the output circuit.

edges of the input image are sensed. Thus, the system operates in a time-sequential and semi-parallel mode. The processing time (that is, frame rate) is $400 \mu\text{s}$. The edge-extraction performance is depicted in Fig. 1.

The second example is an image recognition system using a neural network as a post-processor of the artificial retina output. As shown in Fig. 2f, all detectors are set to a uniform sensitivity of one. The photocurrents (proportional to light intensity) in each VSPD column are summed such that the output current vector, J , constitutes a vertical projection of the input image. Output signal J is a compressed representation of the input image W and is fed into the neural network classifier for recognition. This mode of operation has the advantage that the projection can be obtained in one cycle equal to the VSPD response time of $3 \mu\text{s}$.

We have applied this system to optical character recognition, and have succeeded in recognizing 46 (Japanese) Hiragana characters with almost 100 per cent recognition rate. Furthermore, we have demonstrated a system that can recognize the full set of 1,945 Joyo Kanji characters by feeding the horizontal and vertical projections of the characters into a shift-tolerant neural network classifier.

The application fields of RETINA2 include automotive and avionics sensors, fac-

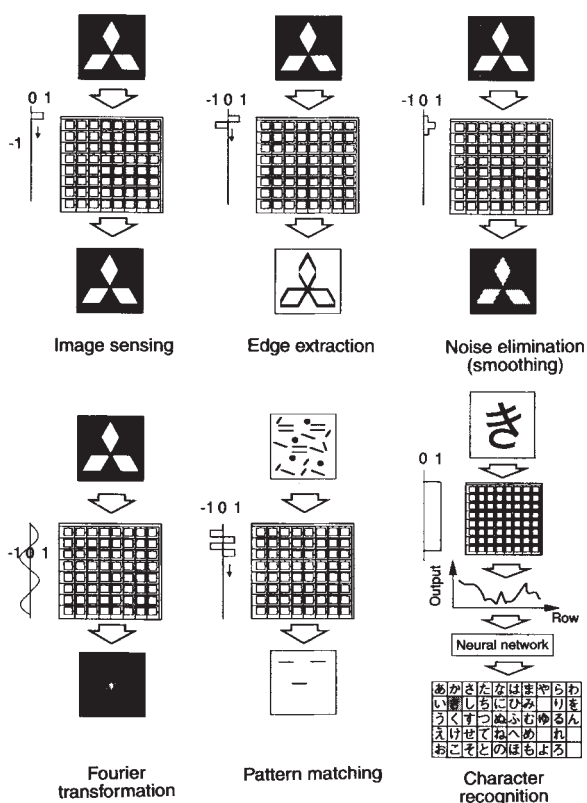


FIG. 2 Examples of image processing options in RETINA2. In the image sensing mode, *a*, all VSPD rows are successively scanned with a single polarity voltage pulse, one at a time. The photocurrents are read out on all column lines simultaneously. Reading out N^2 pixels thus takes merely N clock cycles. To switch to other operating modes one needs only to alter the scanned voltage pattern. For edge extraction, *b*, two adjacent rows are addressed with opposite polarity. The smoothing operation *c* uses a voltage sequence (0.5, 1, 0.5) to suppress noise and smooth out edges. Addressing the VSPD rows with a sinusoidal control signal of wave vector k computes the corresponding k -vector component of the spatial Fourier transform, with one component calculated in one clock cycle. In pattern matching, *e*, the target item is detected by using it as the scanned control signal. Finally, character recognition is facilitated by performing a projection, *f*. Here, all rows are simultaneously addressed with the same voltage, and the entire operation takes only one clock cycle. The retina output (projection) is then fed into a simple neural network for classification.

tory automation and robotics, video still camera, and defence applications (see Fig. 3). One particularly interesting use is as an artificial eye in moving vehicles where it can assist the driver by recognizing road markers. With its particularly fast response, RETINA2 could prevent collisions by sensing obstacles and approaching vehicles.

Retinas in silicon technology

If artificial retinas are to find acceptance outside the laboratory, it is imperative to make use of the low-cost, high-resolution and yield of silicon technology, which furthermore provides ease of integration with a wide selection of processing electronics on the same chip. We are currently building two such retinas. The first is based on

sensing and processing but also learning capabilities to extract features from the images in the real world. In order to realize such 'learning vision chips', we have recently fabricated RETINA3, consisting of a 2-D VSPD array vertically integrated on a one-dimensional LED array^{3,10}. In preliminary experiments, image addition, subtraction and tracing of moving objects have already been achieved using the analog storage function of VSPDs. Furthermore, we have proposed an optical associative memory with learning capability consisting of SLMs integrated on the 2-D VSPD array^{11,12}. The light transmittance of the SLM is adaptively varied in the learning process. Computer simulations indicate that the incomplete images W are successfully transformed into the corresponding point

MOS (metal-oxide-semiconductor) imager cells augmented by on-chip electronics. The second employs high-gain, bipolar npn VSPDs (lateral three-layer structures with donor/acceptor/donor doping, respectively) along with compact CMOS (complementary metal-oxide-semiconductor) addressing electronics. Both display high photosensitivity, dense integration and frame rates which leave plenty of room for post-processing, should it be desired, while still completing the task in real time.

Next generation

More applications will open up as next generation chips combine not only image

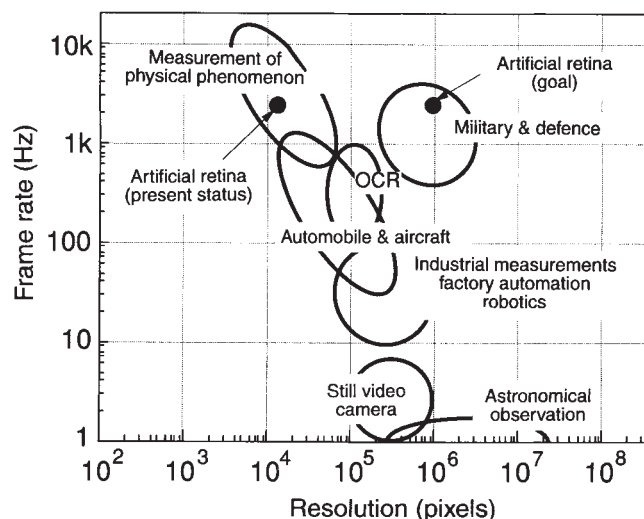


FIG. 3 Performance of the artificial retina and typical requirements in potential areas of application. It is hoped that improved resolution, to be achieved using silicon technology currently under development, will greatly enhance the scope of problems that can be addressed with the artificial retina.

attractors by the nonlinear thresholding of the output J of the VSPD array, and by feedback to the input S .

In summary

Artificial retinas execute multiple, easily programmable forms of image processing with inherently high speed and parallelism. A new generation of high-resolution retinas that incorporate light-emitting devices will further boost processing capabilities. □

Kazuo Kyuma, Eberhard Lange, Jun Ohta, Anno Hermanns, Bryan Banish and Masaya Oita are in the Semiconductor Research Laboratory, Mitsubishi Electric Corporation, 8-1-1 Tsukaguchi Honmachi, Amagasaki, Hyogo 661, Japan. For more information on the artificial retina systems featured in the article, fill in reader service number 100.

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Neuroscience news

New in the neuroscience field — Immunotoxins for use in immunolesioning, a rapid solution changer for patch-clamp experiments, a system for studying the behavioural patterns of animals and a standalone confocal software package.

CHEMICON is offering **immunotoxins for use in immunolesioning** (*Reader Service No. 101*). An immunotoxin is a conjugate between an antibody specific for a cell-surface protein and a protein that is able to disrupt cell function and cause cell death. The combination of these two functions allows the targeting of a specific cell type and results in specific cell elimination. In the case of the immunotoxin system provided by Chemicon, the antibody binds to the cell-surface protein and internalizes saporin, a ribosome-inactivating protein that inactivates cellular protein synthesis and causes cell death. The removal of a specific subtype of neurons allows the investigation into the function and behavioural effects of those neurons. The neuroimmunotoxins available are 192-IgG-SAP, specific for the low-affinity neurotrophin (p75) receptor; OX7-SAP, specific for the rat and mouse Thy 1 antigen; and anti-DBH-SAP, selective for adrenergic and noradrenergic neurons in the central nervous system and peripheral nervous system. Custom immunotoxins can also be provided using your antibody.

A new **radioimmunoassay kit for the determination of melatonin** — a neuroendocrine hormone that modulates annual and circadian biorhythms — in biological fluids is available from Büllmann Laboratories and should provide a useful tool in the fields of clinical neuroendocrinology, chronobiology and psychiatry (*Reader Service No. 102*). The assay is designed to

provide a high sensitivity (0.3 pg ml^{-1} , estimated dose at 50 per cent binding = 6 pg ml^{-1}). And, depending on the sample dilution, a measuring range of $1\text{--}100 \text{ pg ml}^{-1}$ or lower may be used. Büllmann Laboratories says that the test may prove useful for measuring melatonin in cases where a reliable determination has, to date, proved cumbersome, for example, when measuring intraday variations of melatonin secretion, when using small sample volumes (from rodents), and in the *in vitro* secretion of melatonin from cell cultures. Samples are extracted using a reversed-phase cartridge extraction. The assay is based on an overnight incubation and a double antibody separation. The kit comes with two RIA controls. Five prediluted calibrators and ready-to-use reagents are designed to reduce the likelihood of handling errors.

Sigma Immunochemicals introduces seven new **growth factor ELISA kits** for the quantitative detection of human cytokines in culture media, serum, plasma and other



Seven new kits for the quantitative determination of human cytokines.

biological fluids (*Reader Service No. 103*). The line-up includes human interleukin-1 α , human interleukin-1 β , human interleukin-2, human interleukin-4, human interleukin-7, human tumour necrosis factor- α and human tumour necrosis factor- β . The kits feature a 96-well format with removable strips pre-coated with monoclonal primary capture antibody, peroxidase labelled specific secondary antibody, easy reagent preparation for the detection of picogram quantities of growth factor, and results in 5 h or less.

Antibodies

Available from Serotec — a panel of **monoclonal antibodies for the determination of binding domains of fibronectin molecules** (*Reader Service No. 104*). Serotec's panel is designed to offer a comprehensive selection of antibodies and differentials between the three types of fibronectin and to determine the binding domains of the fibronectin types.

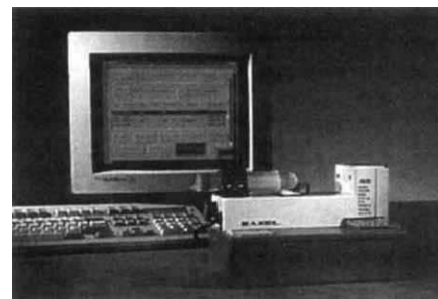
All monoclonals are provided as purified tissue culture supernatant and are intended for use in cell adhesion studies.

Genzyme Diagnostics has introduced a range of new **antibodies with minimal cross-reactivity for therapeutic drug monitoring and drugs of abuse** (*Reader Service No. 105*). The therapeutic drug range includes digoxin, theophylline, phenobarbital, caffeine, vitamin B₁₂ and vancomycin. In terms of drugs of abuse, the antibodies on offer are benzoylecgonine, cocaine and THC. Purified from ascites, all the antibodies are provided in bulk by Genzyme's subsidiary Medix Biotech.

Solution delivery

In patch-clamp experiments, the RSC-100 **rapid solution changer** from Bio-Logic enables rapid, multiple and programmable solution exchange at the pipette tip (*Reader Service No. 106*). The experimental solutions to be assayed are contained in standard plastic syringes and are driven by gravity through plastic tubing to the rotating head (different models with 6, 9, 18, etcetera, tubes are available). The solution exchange is said to be performed by a very precise rotation of the RSC-100 head which exposes the patch pipette or the cell to the flow of a chosen tube (all other tubes can be closed by the electrovalve system). Two adjacent tubes can be switched within 10 ms, says Bio-Logic. The system is designed to be free from dead volume and contamination problems as the solutions are completely separated down to the outflow. Solution changes can be performed manually, by analog pulses from any stimulator or D/A board, or from the computer. The system can be installed in most electrophysiological setups.

The Razel 99, available through Semat Technical, is a high-precision **infusion syringe pump** which accepts standard glass or plastic syringes with capacities that range from 50 cc to microlitres (*Reader Service No. 107*). A choice of 99 delivery speeds is



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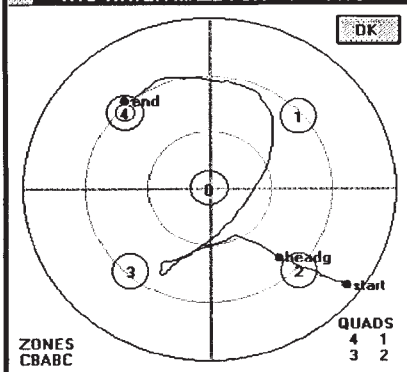
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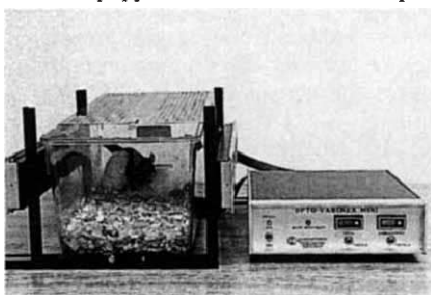
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offered. The motor is controlled either by an electronic circuit, or, using the pulser software, by an IBM or compatible personal computer. With the pulser program, parameters such as type of syringe, starting volume, initial flow rate, volume or time factors can be entered. Once selected, the program will activate and a graph indicating the current volume in the syringe and the time left in each sequence will be presented on screen; hard copies of each sequence can be printed for record keeping. The pump can also be supplied with a reversing feature or with restraints to pull the plunger out of the syringe so that withdrawals may be accomplished. With maintenance in mind, all critical parts are fabricated from stainless steel with bronze bearings. Optional extras include alarms, remote control outputs and an interconnection for a pulse counter.

■ Behavioural studies

A new version of EthoVision, a system for automatic recording of activity, movement and behavioural patterns of animals, is now available from Noldus Information Technology (Reader Service No. 108). Version 1.5 includes significant improvements in the areas of data acquisition and analysis. The program now supports the use of colour CCD cameras and frame grabbers; this permits the tracking of up to eight animals in a single enclosure. Alternatively, up to eight arenas can be observed with a single camera. Version 1.5 can detect various behaviour patterns, for example, the onset and offset of movement or rearing in rodents, as well as interactions between pairs of animals (approach, avoidance, contact, parallel movement). Several new parameters are available to describe the shape of spatial tracks: compass angle and angular deviation express the direction of movement relative to the north or a user-defined reference axis, respectively; meander and sinusity quantify the amount of turning relative to the path length. The full configuration includes EthoVision software and documentation, video camera and monitor, frame grabber, DOS computer, VCR (optional), cables and connectors.

The laboratory animal activity meter from Columbus Instruments which works with standard animal cages for rats or mice is now equipped with IBM PC/AT-compat-

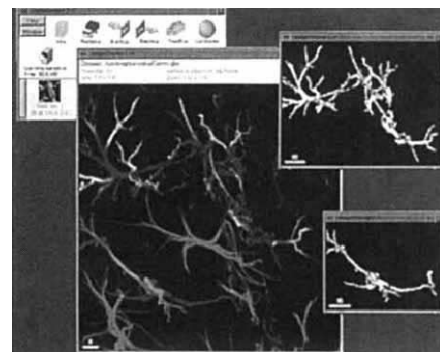


Activity meter — useful for screening medical and chemical substances for neurotoxic effects on animals.

ible data collection software that meets good laboratory practice (GLP) standards (Reader Service No. 109). Each meter can work alone with animal activity figures displayed on front panel counters, or as part of a multi-cage system with data collected by an AT-compatible computer. One front panel counter displays total animal activity including relocations, scratching and grooming, a second displays only information related to ambulatory activity. Non-ambulatory activity can be obtained by subtracting ambulatory activity from total activity. Animal activity is monitored by a set of 15 infrared beams spaced about an inch apart which cross the animal cage from side to side. Separation of animal ambulatory activity from total activity is done by the patented method of counting only sequential beam interruptions and rejecting interruptions that are caused by an animal repetitively interrupting the same beam. Infrared sensors are mounted on an adjustable frame that can accommodate small and large cages. The intensity of the infrared beams is said to be sufficient to penetrate 30 cm of water — making the system suitable for monitoring the activity of fish in a small aquarium.

■ Software

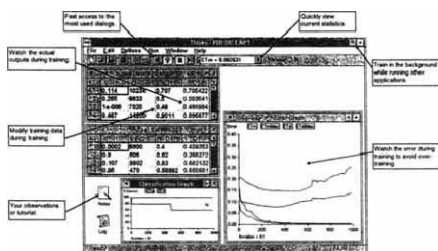
ImageSpace 3.1 is a confocal software program available from Molecular Dynamics that is designed for three-dimensional (3-D) visualization and quantitation (Reader Ser-



ImageSpace — confocal software from Molecular Dynamics — is now available as a standalone package.

vice No. 110). The program, previously only available as part of the company's confocal laser scanning microscope systems, is now being offered as a separate product complete with import routines for images from all confocal systems. It provides an integrated environment for rendering, processing, analysis and display of confocal images. The program, which runs on Silicon Graphics' workstations, enables the researcher to perform quantitative image analysis on 3-D data. Analytical capabilities include automated object counting, seeding, segmentation, and tools for quantitative colocalization studies.

Logical Designs Consulting announces the release of THINKS neural network
NATURE · VOL 372 · 10 NOVEMBER 1994



THINKS — neural network training application software.

training application software for Microsoft Windows 3.1 (*Reader Service No. 111*). The program integrates neural network algorithms with a user-friendly Microsoft Windows interface. The use of a tool bar puts most dialogues just one click away and the entire manual is online in the help file. With many options on network architecture and processing element definition, the experienced user can quickly experiment with novel network configurations, says the company. The product line also provides a migration path for those wishing to develop custom applications. By offering the core neural network functions in several forms, including DOS run time libraries for various compilers, a Windows 3.1 dynamic link library, and documented source code portable to any environment, the user can develop any neural network application.

■ Image analysis

The new AxioHOME computer-assisted microscope from Carl Zeiss is designed to improve reliability in medical diagnosis (*Reader Service No. 112*). A small video monitor is integrated into the system, allowing a computer display to be superimposed onto the microscope image seen through the eyepieces. This enables many measuring, drawing and masking tasks to be performed interactively, using general morphometry software. The nosepiece and the stage of the microscope are both coded, so that the computer not only recognizes the magnification of the objective in use, but electronically tracks and stores every movement of the stage. This is said to provide precise and reliable relocation of individual specimen areas with their associated overlay data, at the correct magnification, for further examination or for quality control checks. The 'drawing' measurements and other relevant data is stored in the computer system and can be recalled when the slide is reloaded for further examination. The system, which was developed with the cooperation of pathologists from the pathology departments at the University of Edinburgh and St Bartholomew's Hospital, London, may be used, for example, to measure the diameters of striated muscle fibres in neuromuscular disease.

The MiraCal **ratiometric imaging system** from Life Science Resources uses a small area, cooled read-out charge coupled

device (CCD) camera with which the capture time can be computer-controlled from milliseconds to seconds (*Reader Service No. 113*). The optimized area of the camera (192×165 pixels) is designed to reduce the amount of information being processed, and allows for a read-out time of about 30 ms into the controlling computer. Depending on the amount of light available to the CCD, acquisition rates of up to 4 fps are possible, according to the company, and the exposure time of the CCD can be increased to improve sensitivity at the expense of acquisition speed. Such acquisition rates are said to be



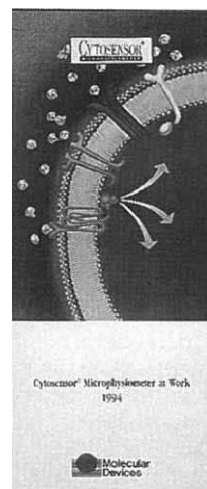
MiraCal system showing a normal 35-mm camera on the front port of the microscope and the cooled CCD detector on the side.

sufficient for most biological experiments where cellular events happen over timescales ranging from seconds to minutes. In addition, the company says that the fine control over the sensitivity allows light output from different wavelengths to be matched by varying the exposure given at each wavelength. The MiraCal system includes a microscope, filter wheel, high-resolution graphics controller, computer and camera. At the heart of the system is the computer software used to control acquisition and analysis of ratiometric data. It allows for the capture of data at up to four wavelengths per experiment, allowing experiments with multiple dyes to be performed.

■ Reference guides/catalogues

Listed in a recent reference guide to the **microphysiometer**, available from Molecular Devices and entitled *Cytosensor*

Microphysiometer at Work, are recent scientific publications and poster presentations published using the system for drug discovery and signal transduction research (*Reader Service No. 114*). The guide is intended to illustrate the versatility of the system for studying ligand/receptor interactions from a variety of receptor families. These include G-protein linked receptors, tyrosine kinase receptors and ligand-gated ion channels. For each type of receptor and effector agent used in the system, scientific references are listed along with the cell types used.



Reference guide

The latest 1994–95 **physiology research instruments catalogue** from Stoelting includes a range of instruments for physiology, pharmacology and neuroscience — indeed any whole animal research laboratory (*Reader Service No. 115*). Featured among the 80 pages are products that include micropipette pullers, micromanipulators, stereotaxic instruments, swivels, infusion/withdrawal syringe pumps and the MacLab data acquisition system. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. For more details on any of the products featured, fill in the reader service card bound inside the journal.

CORRECTION

In the new products for VEGF research that appeared in the 22 September issue (*Nature* 371, 362; 1994), the phrase "the antibodies were raised against the 121 variant" should have read "the antibodies were raised against the 165 variant".

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Receptor malfunction

The family of four fibroblast growth factor receptors is stimulating attention with the discovery of genetic defects tying three members to a variety of congenital bone malformations.

THE field of bone morphogenesis has undergone a revolution in the past few months with the discovery that no fewer than three members of the fibroblast growth factor receptor (FGFR) gene family are, when defective, involved in a variety of hereditary disorders of bone development, including achondroplasia, the most common form of human dwarfism. In each case, genetic mapping has rapidly led to the isolation of the genes responsible for achondroplasia^{1,2}, Crouzon syndrome³ and now, as described in this month's issue of *Nature Genetics*, Pfeiffer syndrome⁴.

The first FGFR defect was found earlier this year, following the mapping by several groups of the locus for achondroplasia to the distal end of the short arm of chromosome 4. A candidate gene, *FGFR3*, lurking near the achondroplasia locus, had been examined but quickly discarded during the ten-year hunt for the Huntington's disease gene, but genetic analysis quickly showed that patients with achondroplasia harboured an identical missense mutation in the region of the gene encoding the transmembrane domain^{1,2}. This finding was all the more remarkable given the very high *de novo* rate of achondroplasia mutations.

At about the same time, Robin Winter and colleagues at the Institute of Child Health, London, discovered the gene for Crouzon syndrome³, one of dozens of different malformations known as the craniosynostoses. These are disorders of variable severity in which the cranial sutures fail to fuse during development, leading to malformed skulls that often require major surgery; occasionally, there are also distal limb defects. Winter's group found that mutations in an alternatively spliced exon of *FGFR2* on chromosome 10, encoding an extracellular immunoglobulin-like domain, are responsible for many cases of Crouzon syndrome.

Sensing something of a trend, Maximil-

lian Muenke (of the Children's Hospital, Philadelphia, and working in collaboration with Winter's group) realized when he mapped to chromosome 8 the locus for yet another related disorder, Pfeiffer syndrome, that the logical candidate in the area had to be the gene for a third FGFR molecule, *FGFR1*. In five apparently unrelated families with Pfeiffer syndrome, Muenke *et al.*⁴ have discovered that the same mutation (a substitution of arginine for proline at residue 252), again in the extracellular portion of *FGFR1*, segregates with the disease.

Interestingly, Crouzon syndrome has just been shown to be allelic with Jackson-Weiss syndrome, which is characterized by hand and feet abnormalities as well as cranial disorders. Having studied the Amish kindred in which Jackson-Weiss syndrome was originally described, Jabs *et al.*⁵ now report the finding of a mutation that lies just two residues away from one of the Crouzon syndrome substitutions.

These remarkable discoveries are certain to improve greatly our understanding of the developmental role of fibroblast growth factors and their receptors, studies of which until now have been largely confined to such lower organisms as *Drosophila* and *Xenopus*⁶. It is unlikely that progress will stop here: there are four known mammalian FGFRs and nine different fibroblast growth factors, suggesting that careful scrutiny of the loci for these factors and other dysmorphological syndromes will soon herald many fascinating discoveries.

A similar strategy to find candidate genes has also paid off handsomely in unravelling the molecular basis of a form of dystonia, as described elsewhere in this month's issue of *Nature Genetics*. Toshiharu Nagatsu and colleagues at Fujita Health University in Japan report the identification of four different mutations in the gene for GTP cyclohydrazase I in patients with hereditary progressive dystonia⁷. This disorder, also known as DOPA-responsive dystonia, is characterized by a progressive movement disorder similar to Parkinson's disease, and was recently mapped to chromosome 14. The enzyme GTP cyclohydrazase I catalyses a rate-limiting step in the biosynthesis of biopterin, a crucial cofactor for several different enzymes involved in the manufacture of dopamine. Ichinose *et al.*⁷ have mapped

the GTP cyclohydrazase I gene to chromosome 14, and show that four patients with DOPA-responsive dystonia carry mutations in this gene; in at least two cases these mutations completely destroy the activity of the enzyme.

Elsewhere in Japan, researchers at Kyoto University led by Akira Kikuzuka have uncovered another example of an expansion of a CAG trinucleotide repeat, in this instance one responsible for the neurodegenerative condition Machado-Joseph disease⁸. The gene encodes a protein of 359 amino acids that has no familiar features other than the putative polyglutamine stretch of 13 to 36 residues towards the carboxy-terminal end. But in individuals affected with this dominant progressive disorder, which bears many similarities to Huntington's disease and spinocerebellar ataxia (SCA), this segment has as many as 79 repeats.

Five loci for spinocerebellar ataxia have now been genetically mapped in humans, thanks to the discovery by Ranum *et al.*⁹, reported in this issue, of the chromosomal assignment of SCA5. This may seem fairly unremarkable in itself, but the same cannot be said for the pedigree that enabled the gene to be mapped: the trait was evidently passed down by the paternal grandparents of President Abraham Lincoln. Two of the president's father's siblings inherited the errant gene for SCA5, and study of their extended families has allowed Ranum *et al.* to localize the gene to chromosome 11.

President Lincoln was the subject of genetical speculation a few years ago: when the gene for Marfan syndrome was isolated, it was suggested that he might have suffered from this condition. He was assassinated at the age of 56 and has no living direct descendants. But noting that SCA5 is a fairly mild, late-onset form of ataxia, Ranum and colleagues cannot resist asking whether the president might perhaps have inherited the faulty version of this gene too.

Kevin Davies

Kevin Davies is Editor of *Nature Genetics*.

Also in this month's *Nature Genetics*: a new syndrome involving interchange of the long arms of the X and Y chromosomes; mutations in the human homologue of microphthalmia cause Waardenburg syndrome type 2; CGG triplet repeat expansion in FRA3F; a possible locus for bipolar affective disorder; and identification of the Xg blood group.

1. Shiang, R. *et al.* *Cell* **78**, 335-342 (1994).
2. Rousseau, F. *et al.* *Nature* **371**, 252-254 (1994).
3. Reardon, W. *et al.* *Nature Genet.* **8**, 98-103 (1994).
4. Muenke, M. *et al.* *Nature Genet.* **8**, 269-274 (1994).
5. Jabs, E.W. *et al.* *Nature Genet.* **8**, 275-279 (1994).
6. Mason, I. J. *Cell* **78**, 547-552 (1994).
7. Ichinose, H. *et al.* *Nature Genet.* **8**, 236-242 (1994).
8. Kawaguchi, Y. *et al.* *Nature Genet.* **8**, 221-228 (1994).
9. Ranum, L. P. W., Schut, L. J., Lundgren, J. K., Orr, H. T. & Livingston, D. M. *Nature Genet.* **8**, 280-284 (1994).

Changing times for neuroscientists

Diane Gershon

At a time of considerable uncertainty for the biotechnology industry, *Nature* reviews the prospects for neuroscientists considering a move out of the academic sector.

Houston. These are not the best of times for the biotechnology industry. Faced with some very public product failures this year and an acute shortage in the availability of capital, biotechnology companies are having to bite the bullet, cut costs and refocus their research and development (R & D) efforts until the public markets re-open. In the area of neuroscience, specifically, there has been a dearth of breakthrough products and, so, almost five years into the Decade of the Brain, the potential payoffs from basic research are still showing more promise than progress.

Climates

The prospect of health-care reforms loomed large this year. This, coupled with the failure of some products to meet expectations in clinical trials, sent investors on Wall Street running for the hills and left biotechnology companies out in the cold. Public stock offerings took a beating, particularly in July and August.

And then the Blech bombshell hit. Stocks in the many fledgling biotechnology companies that were taken public by David Blech's New York brokerage firm, D. Blech & Co., took a tumble in late September when the firm announced it had suspended making markets in biotechnology stocks after its financial reserves had dipped below the level required by the US Securities and Exchange Commission. Although another brokerage firm has since stepped up to the plate and agreed to buy Blech's customer accounts, many analysts feel that it will be some time before Blech's stocks recover, if at all.

In order to weather what has been an extended dry financial spell, some companies have had to respond by instituting cost-cutting measures — layoffs or restructuring, or both. As a sign of the times, Alkermes Inc. of Cambridge, Massachusetts, which specializes in the development of novel drug delivery products targeted at the central nervous system, announced in September plans to cut its workforce by 25 per cent.

By the end of September there were signs that the market was picking up when it became clear that there was almost no chance that the US Congress would pass a health-care reform bill this session.

So the name of the game this year has been to find ways to remain "visible and viable", in the absence of having — in some cases — products, says John Groom, president and chief executive officer of

Athena Neurosciences Inc. of South San Francisco, California (one of 20 or so 'neuroscience' companies in the United States). "We've tried to pull ourselves up by our bootstraps", says Groom, who expects his company to achieve profitability — sooner than most — in 1996. Then, he says, the company would not be so dependent on the vagaries of financing. The company is already generating income having acquired a product for the treatment of Parkinson's disease from Eli Lilly & Co. and by developing a 'mail-order' pharmacy business for neurologists.

Frederick J. Dorey, president of the Bay Area Bioscience Center, an information clearinghouse for the industry located in Oakland, California says that the golden rule of biotech is to go for the "home runs" — diseases for which there are currently no effective therapies. In neuroscience, "that's a tough haul because it's such a complex and difficult area".

Hiring practices

Even during these austere times, there are jobs to be had. The days of speculative recruitment are long gone, having shifted to more focused and specialized recruitment strategies. Hiring practices vary widely. Word-of-mouth recommendations, international, national and local advertising, and the use of recruitment 'executive' search firms are all used to a lesser or greater extent.

Companies such as Massachusetts-based Cambridge NeuroScience Inc. favour the placement of advertisements in leading science journals. The company also trows locally by running the same advertisement in the *Boston Globe*.

The nonprofit Bay Area Bioscience Center posts up-to-date listings on job opportunities in northern Californian biotechnology companies but the centre lacks the resources either to sort jobs by scientific discipline or to send the information out upon request. "There's a quantum leap in trying to get into a more detailed focus," says Dorey, although the centre hopes in the future to make the information available online.

The use of recruitment agencies, or 'head hunters' seems to be popular among some of the larger firms with deeper financial pockets. Increasingly, they are being used to identify candidates for senior-level technical and scientific posts, and not just for executive-type positions.

Some see head hunters as unnecessary

intermediates. Using recruitment firms "isn't the best way to go on a cost-per-hire basis and, more importantly, on a quality basis", says Ross Gibson, senior director of corporate resources at Cambridge NeuroScience. Gibson says that his company will resort to their use only when filling some of the more "needle in a haystack-type positions", such as an MD/PhD neurologist.

Marvin R. Brown, chief executive officer of Alanex Corporation, San Diego, California, a start-up company focusing on the design of small-molecule drugs that act through peptide receptors, says that he would do almost anything to avoid using them. Most of the company's recruitment to date has been in the area of chemistry using the placement of advertisements in *Chemical & Engineering News*.

Stephen J. Peroutka, president of the neuroscience start-up company, Spectra Biomedical Inc. of Menlo Park, California, says it can be useful, particularly in small companies (Spectra has 20 employees — not all full-time), initially to offer people contract work and pay on a 'per-hour basis' before making a decision on whether to take them on full-time.

Apart from the recruitment of specialists, it is "a bit of a treadwater time" for biotechnology companies, says Athena Neurosciences' Groom. In his experience, there continues to be a scarcity of good pharmacologists and medicinal chemists "but you can buy molecular biologists by the yard". And, because of the density of biotechnology companies in the San Francisco area, good research associates and technicians — especially those with some experience — can be hard to recruit. Groom also finds recruiting people with pharmaceutical experience to be problematic because invariably it means relocating the person from the East Coast where most of the companies are situated.

Making the transition

Given the current funding difficulties, academic scientists seem more receptive than they used to be to taking a job in industry. "It used to be that universities represented low risk, high security, but that's changing," says Alanex's Brown. Brown, who co-founded the company in 1991 and who still holds an adjunct appointment as professor of medicine at the University of California, San Diego, says that attracting the right calibre of people under the same roof all buying into

US BIOTECHNOLOGY COMPANIES WITH SUBSTANTIAL NEUROSCIENCE R & D PROGRAMMES

Therapeutics/Diagnostics

Alanex Corporation, San Diego, CA

Status: privately held

Corporate interests: use of computational molecular design and synthetic chemistry to develop small-molecule, non-peptide drugs that either mimic or inhibit the actions of peptides. The company is developing a new class of molecules based on the neurotransmitter Neuropeptide Y. Collaboration with Amgen Inc.

Athena Neurosciences Inc., South San Francisco, CA

Status: publicly held

Corporate interests: development of therapeutic and diagnostic products for neurological diseases and disorders, with a leading research effort in Alzheimer's disease in collaboration with Eli Lilly. In the field of brain inflammation, the company is focusing on developing treatments for head injury, multiple sclerosis and stroke.

Cambridge NeuroScience Inc., Cambridge, MA

Status: publicly held

Corporate interests: development of ion-channel blockers for the treatment and prevention of brain damage resulting from stroke, traumatic brain injury and surgery, and glial growth factors for the treatment of peripheral neuropathies and degenerative muscle disorders, such as muscular dystrophy.

Cephalon Inc., West Chester, PA

Status: publicly held

Corporate interests: development of treatments for neurodegenerative diseases and disorders such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease and narcolepsy.

CoCensys Inc., Irvine, CA

Status: publicly held

Corporate interests: development of CNS drugs to treat neurological and psychiatric disorders such as epilepsy, anxiety and sleep disorders, and cerebral ischaemia caused by stroke and head injury. Alliance with Ciba Pharmaceutical.

Cypros Pharmaceutical Corporation, Carlsbad, CA

Status: publicly held

Corporate interests: using an "in-licensing" approach, the company has a drug in clinical trials for the treatment of stroke and/or traumatic head injury. Research involves development of an adenosine metabolism inhibitor, neuronal calcium channel and glial chloride channel blockers.

Geron Corporation, Menlo Park, CA

Status: privately held

Corporate interests: development of drugs for the treatment of age-related degenerative diseases and cancer.

Giltech Inc., Cleveland, OH

Status: privately held

Corporate interests: based on its glial cell research, the company is developing therapeutic products with potential applications in areas such as the treatment of Alzheimer's disease, anxiety and coma. Giltech last month announced plans to collaborate with Janssen Pharmaceutica N.V.

Neurex Corporation, Menlo Park, CA

Status: publicly held

Corporate interests: using a targeted licensing-in strategy, as well as its own discovery programmes, the company is building a portfolio of products in the acute care area. Neurex's main discovery effort is focused on a proprietary neuromodulation technology, which enables the selective blocking of different classes of neuron-specific calcium channels, thereby regulating the release of specific neurotransmitters.

Neurogen Corporation, Branford, CT

Status: publicly held

Corporate interests: development of products for the treatment of neuropsychiatric disorders. Five-year R & D agreement with Pfizer Inc. in the GABA receptor area for the development of drugs to treat anxiety.

Regeneron Pharmaceuticals Inc., Tarrytown, NY

Status: publicly held

Corporate interests: use of neuronal growth proteins for the treatment of degenerative and traumatic neurological disorders, such as Alzheimer's, Parkinson's and Huntington's diseases and ALS.

SIBIA Inc., La Jolla, CA

Status: privately held

Corporate interests: development of therapeutics for neurodegenerative diseases and other CNS conditions, including Alzheimer's disease and other dementias, epilepsy, stroke and Parkinson's disease. Corporate partnerships with Eli Lilly & Co. and Ciba-Geigy Ltd.

Signal Pharmaceuticals Inc., San Diego, CA

Status: privately held

Corporate interests: development of small-molecule therapeutics that regulate the transcription factors involved in the progression of a number of inflammatory, viral and neurological diseases. Disease

targets include Alzheimer's and Parkinson's disease, stroke, trauma and ALS.

Spectra Biomedical Inc., Menlo Park, CA

Status: privately held

Corporate interests: development of diagnostics and therapeutics based on an understanding of the genetics of human diseases and traits. The company's initial focus is on neuropsychiatric disorders, particularly migraine, anxiety and depression. Collaborative research agreement with Glaxo Holdings plc in the field of migraine therapeutics.

Sugen Inc., Redwood City, CA

Status: privately held

Corporate interests: receptor-based drug discovery and development with a primary emphasis on the tyrosine kinases and phosphatase receptor systems.

Synaptic Pharmaceutical Corporation, Paramus, NJ

Status: privately held

Corporate interests: discovery of novel G-protein-coupled receptors expressed in the CNS.

Synergen Inc., Boulder, CO

Status: publicly held

Corporate interests: development of neurotrophic factors for the treatment of degenerative neurological diseases and conditions such as ALS, Parkinson's and Alzheimer's diseases. Neuroscience joint venture with Syntex (USA) Inc.

Drug delivery

Alkermes Inc., Cambridge, MA

Status: publicly held

Corporate interests: development of drug delivery technologies. The company's leading product candidate, a receptor-mediated permeabilizer, is being developed to improve the treatment of brain tumours and infections of the CNS.

Alza Corporation, Palo Alto, CA

Status: publicly held

Corporate interests: development of therapeutic systems of drug delivery. In the neuroscience area, Alza is concentrating on disorders of the CNS (e.g. pain management, psychiatry and neurology).

CytoTherapeutics Inc., Providence, RI

Status: publicly held

Corporate interests: development of implantable delivery systems for biologically active and gene therapy products for the treatment of CNS diseases and other chronic disorders.

This is not a comprehensive list but instead provides a guide to the number and types of companies in the neuroscience area. Other biotechnology companies such as Amgen Inc. and Genentech Inc. have neuroscience programmes, as do most US pharmaceutical companies.

the same idea is tough to do in academic institutions.

Switching fields can also be tricky in an academic setting. A PhD pharmacologist by training, Peroutka says that it would have been impossible for him to get federal funding to move from pharmacology to genetics. "Industry allows you to really go where you need to go scientifically and provides the resources to do that in a way that's just not possible in academia." Peroutka, a former director of neuroscience at Genentech Inc., founded Spectra Biomedical last year to identify the molecular genetic basis of migraine and other neuropsychiatric diseases such as anxiety and depression.

For those academic scientists who are taking a hard look at a career in biotechnology, the attractions are plain to see: an

end to grant writing and the often heavy administrative burdens of faculty life. The option of owning stock in a company can't hurt either. It also provides the opportunity to be a team player in an entrepreneurial enterprise and the chance to see something practical evolve from their research efforts. The downside is the high degree of risk (the company could go 'belly up'), but with risk comes the potential for reward, says Gibson.

The question of academic freedom — freedom to publish, attend scientific meetings and maintain close academic ties — is a commonly voiced concern among scientists contemplating a move. Company policy may vary on this but, at best, researchers should expect to experience some delays when publishing.

Many companies encourage employees

to foster academic collaborations. And, although Athena Neurosciences' Groom encourages his employees to do likewise, he draws the line at them holding adjunct appointments (with the exception of physicians, whom he says should maintain some 'hands-on' experience with patients). "Having someone with one foot in academia and one foot in industry is not good", he says, particularly as some scientists find it hard enough to break with academic tradition.

In the end, those who succeed in industry are the ones who are most comfortable working in an environment where change is constant, says Gibson. As Peroutka puts it, scientific adaptability is the key. □

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